

Bulletin No 52 from the Department of Surgery,

University of Lund, S-221 85 LUND

1985

BILIARY SEPSIS

An experimental study in rat

with special reference to host defense failure

in biliary obstruction



Nobutaka Tanaka



BILIARY SEPSIS

An experimental study in rat
with special reference to host defense failure
in biliary obstruction

Nobutaka Tanaka

leg läk, Ld.

Akademisk avhandling

som för avläggande av doktorsexamen i medicinsk vetenskap

vid Medicinska Fakulteten vid Universitetet i Lund

kommer att offentligen försvaras å Föreläsningssal 3,

Centralblocket, Lasarettet i Lund,

lördagen den 1 juni 1985, kl 09.00.

Organization LUND UNIVERSITY Department of Surgery University of Lund S-221 85 LUND Sweden		Document name DOCTORAL DISSERTATION	
		Date of issue 1985-06-01	
		CODEN: LUMEDW/(MESL-1031)/1-140/1985	
Author(s) Nobutaka Tanaka		Sponsoring organization	
Title and subtitle BILIARY SEPSIS. An experimental study in rat with special reference to host defense failure in biliary obstruction.			
Abstract A method of retrograde intrabiliary (RI) injection of bacteria which simulates the ascending infection of bacteria from the duodenum was developed in rats. Normal rats immediately after biliary obstruction resisted well RI injection of <i>E. coli</i>. Whereas, an increased susceptibility to RI injection of bacteria was demonstrated by rats with 3 days' biliary obstruction. Animals with longstanding jaundice were more susceptible than with a shorter duration of biliary obstruction. RI injection of <i>E. coli</i> caused an increased mortality in splenectomized rats. Despite active immunization, RI injection of bacteria resulted in a high mortality in chronic biliary obstruction. Using ¹²⁵I-labelled <i>E. coli</i> for RI injection, bacterial clearance from the biliary tree and the uptake rate by the liver was estimated. Bacterial clearance rate from the biliary tree was greatly impaired and the uptake of bacteria by the liver was severely inhibited in rats with chronic biliary obstruction. Bacterial clearance from the blood and organ localization of bacteria was determined by intravenous injection of ¹²⁵I-labelled <i>E. coli</i>. The clearance rate was not impaired in chronic biliary obstruction. A decreased localization of bacteria in the liver per unit weight was detected in chronic biliary obstruction, although a total organ uptake rate in the liver was not decreased in these rats. Active immunization increased the lung uptake of bacteria in chronic jaundiced rats. Reticuloendothelial function in obstructive jaundice was evaluated by measuring the biokinetics of an intravenous injection of a standardized ⁹⁹Tc^m-sulphur colloid. The depression of reticuloendothelial activity of the liver was demonstrated in chronic biliary obstruction.			
Key words Biliary obstruction; biliary sepsis; <i>E. coli</i> ; retrograde intrabiliary injection; reticuloendothelial function; splenectomy; immunization.			
Classification system and/or index terms (if any)			
Supplementary bibliographical information		Language English	
ISSN and key title		ISBN	
Recipient's notes		Number of pages 140	Price
		Security classification	

Distribution by (name and address) Dr. Nobutaka Tanaka, M.D., Department of Surgery, University of Lund, S-221 85 LUND, Sweden

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature Nobutaka Tanaka

Date 1985-04-23

From the Department of Surgery, University of Lund,
S-221 85 LUND, Sweden

BILIARY SEPSIS

An experimental study in rat
with special reference to host defense failure
in biliary obstruction

by
Nobutaka Tanaka

Lund 1985



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41546224_0016

The thesis is based on the following papers. In the text these papers are referred to by their Roman numerals:

- I A RAT MODEL FOR SEPSIS IN CHRONIC BILIARY OBSTRUCTION. Tanaka N, Christensen P, Rydén S, Klöfver-Ståhl B, Bengmark S.
Acta path microbiol scand Sect B in press
- II BILIARY OBSTRUCTION AND SUSCEPTIBILITY TO BILIARY SEPSIS IN RATS. Tanaka N, Christensen P, Rydén S, Klöfver-Ståhl B, Bengmark S.
Res Exp Med 185:115-119, 1985
- III DECREASED CLEARANCE OF ESCHERICHIA COLI FROM THE BILE IN RATS WITH OBSTRUCTIVE JAUNDICE. Tanaka N, Christensen P, Rydén S, Klöfver-Ståhl B, Bengmark S.
Eur Surg Res in press
- IV IMPAIRED LIVER CLEARANCE OF BACTERIA IN RATS WITH CHRONIC BILIARY OBSTRUCTION. Tanaka N, Christensen P, Rydén S, Klöfver-Ståhl B, Bengmark S.
Submitted for publication
- V PROTECTIVE ROLE OF THE SPLEEN AGAINST BILIARY SEPSIS IN RAT. Tanaka N, Christensen P, Rydén S, Klöfver-Ståhl B, Bengmark S.
Submitted for publication
- VI ROLE OF CIRCULATING ANTIBODIES IN BACTERIAL CLEARANCE IN RATS WITH CHRONIC BILIARY OBSTRUCTION. Tanaka N, Christensen P, Rydén S, Klöfver-Ståhl B, Bengmark S.
Submitted for publication
- VII RETICULOENDOTHELIAL FUNCTION IN RATS WITH OBSTRUCTIVE JAUNDICE. Tanaka N, Rydén S, Bergqvist L, Christensen P, Bengmark S.
Submitted for publication

Contents

ABBREVIATIONS.....	8
FOREWORD.....	9
1. BACKGROUND.....	10
1.1. Bacteriology of the healthy liver and biliary tree.....	10
1.2. Bacteriology of the biliary tract in biliary diseases.....	10
1.3. Risk factors for biliary infection.....	12
1.4. Source and route of acquisition of bacteria in biliary sepsis...	12
1.5. Mechanisms leading to biliary sepsis.....	14
1.6. Clinical pictures of septic cholangitis.....	15
1.7. Treatment of biliary sepsis.....	16
1.7.1. Decompression procedures.....	16
1.7.2. Antibiotic therapy.....	17
1.8. Host defense mechanism.....	18
1.8.1. Bacterial killing.....	18
1.8.2. The macrophage system.....	18
1.8.3. Macrophage function.....	19
1.8.4. Opsonins.....	19
1.8.5. Role of the spleen.....	20
1.8.6. RE function in obstructive jaundice.....	20
1.9. Experimental biliary obstruction.....	21
1.10. Experimental models for biliary sepsis.....	21
2. AIMS OF THE PRESENT STUDY.....	23
3. MATERIALS AND METHODS.....	24
3.1. Animals.....	24
3.2. Bacteria.....	24
3.3. Radiolabelling of bacteria.....	24
3.4. Test substance in scintillation camera technique.....	24
3.5. Bacterial vaccine.....	24
3.6. Quantitation of rat IgG antibodies to surface antigens of <u>E. coli</u> 25	25
3.7. Anesthesia and surgical procedures.....	25
3.8. Biliary obstruction.....	25
3.9. Splenectomy.....	25
3.10. Method of retrograde intrabiliary (RI) injection of bacteria...	25
3.11. Bacterial challenge.....	26
3.12. Measurement of bacterial clearance and organ distribution.....	26
3.13. Measurement of RE function by scintillation camera technique and computer analysis.....	27
3.14. Statistical analysis.....	28
4. RESULTS.....	29
4.1. Effect of CBD obstruction in rats.....	29
4.2. Effect of RI injection of <u>E. coli</u> in rats with CBD obstruction..	29
4.3. Intraperitoneal (IP) and intravenous (IV) injection of <u>E. coli</u> ..	33
4.4. Bacterial clearance from the biliary tree.....	34
4.5. Bacterial clearance capacity of the liver.....	35
4.6. Vascular clearance of ¹²⁵ I-labelled <u>E. coli</u>	36
4.7. RE function measured by ⁹⁹ Tc ^m -sulphur colloid.....	37

5.	DISCUSSION.....	41
5.1.	Methodological considerations.....	41
5.2.	RE function in biliary obstruction and biliary sepsis.....	43
5.3.	Clinical considerations and future aspects.....	47
6.	SUMMARY AND CONCLUSIONS.....	48
7.	ACKNOWLEDGEMENTS.....	49
8.	REFERENCES.....	50

ABBREVIATIONS

ALP	: Alkaline phosphatase
ALAT	: Alanine transaminase
AOSC	: Acute obstructive suppurative cholangitis
APT-time	: Activated partial thromboplastine time
ASAT	: Aspartate transaminase
<u>B. fragilis</u>	: Bacteroides fragilis
BSP	: Bromsulphophtalein
CBD	: Common bile duct
CFU	: Colony forming units
<u>Cl. perfringens</u>	: Clostridium perfringens
cpm	: Counts per minute
ERCP	: Endoscopic retrograde cholangio-pancreatography
<u>E. coli</u>	: Escherichia coli
GT	: γ -Glutamyl transpeptidase
Hb	: Hemoglobin
Ht	: Hematocrit
IP	: Intraperitoneal
IV	: Intravenous
MPS	: Mononuclear phagocyte system
PBS	: Phosphate buffered saline
PTBD	: Percutaneous transhepatic biliary drainage
PTC	: Percutaneous transhepatic cholangiography
RE	: Reticuloendothelial
RES	: Reticuloendothelial system
$^{99}\text{Tc}^{\text{m}}$	: Technetium

FOREWORD

Patients with cholangitis, presenting with chills and fever, or shock, are subjected to intensive resuscitative therapy including biliary drainage and antibiotic administration. Despite this vigorous treatment, the condition remains a serious threat to the patient's life with a mortality up to 40 % (1). An appreciation of the pathophysiology of biliary sepsis is still far from complete.

In order to break through the deadlock in the understanding of this disease process, appropriate animal models are used in the hope of providing the key. Studies of the mechanisms behind the susceptibility of the biliary tree to bacterial infection are expected to lead the way forward.

The present thesis was designed especially to elucidate altered host-defense mechanisms in biliary sepsis in rats with biliary obstruction.

1. BACKGROUND

1.1. Bacteriology of the healthy liver and biliary tree

At the turn of this century, healthy dogs were reported to harbour anaerobic bacteria in the liver in the form of Clostridia (2). In 1953, Schweinburg and Sylvester (3) claimed that Clostridia were present in the livers of dogs and rabbits, whereas the liver and biliary tree of rats, guinea pigs and golden hamsters were sterile. The bacteria identified in these early studies were almost exclusively Clostridium welchii, which do not require a specially sensitive anaerobic culture or transport technique. Clostridia are frequently found in the intestinal tract and the skin of the dog. Thus, there exists a possibility in these studies for contamination of samples during collection. Using a more sophisticated technique for anaerobic culture and a larger number of animals, Lykkegaard Nielsen et al. (4) could not demonstrate aerobic or anaerobic bacteria in the liver, gallbladder or bile ducts of healthy rabbits, guinea pigs, mice or pigs. However, the individual biopsy specimens were always small, and the inability to extrapolate any result to include the entire organ has been a serious deficiency. Although an adequate precaution against bacterial contamination can be taken, the possibility of the existence of anaerobic flora in the liver or biliary tree in healthy animals cannot yet be excluded.

Likewise, the existence of the bacterial flora in the normal liver and biliary tree in humans has been a matter of debate. In contrast to the belief that the liver and the biliary tree usually do not harbour bacteria in humans without biliary disease (5, 6), Edlund et al. (7) demonstrated a fairly constant harbouring of anaerobic bacteria in the liver of patients with or without biliary disease. Lykkegaard Nielsen et al. (8), however, claimed that the anaerobic gram positive rods, which were included in the group of anaerobic flora in the study of Edlund et al. (7) might be due to an anaerobic contaminant, such as Propionibacterium acnes. Although taking great precautions with the technique could perhaps prevent bacterial contamination, it may still be difficult to discriminate the anaerobic flora from the contamination. Edlund et al. (7) seems not to accept the concept of anaerobic contamination. On the other hand, Lykkegaard Nielsen et al. (8) emphasized the importance of methods of anaerobic culture and method of transport. But, they seemed to pay less attention on the homogeneity of different groups. It is well known that elderly people in biliary diseases have a propensity for biliary infections (9). Therefore, there exists a possibility that bacteria reside in the liver in the normal aged population. Reports concerning the bacteriology of the biliary tree in healthy subjects are quite few. Using endoscopic retrograde cholangio-pancreatography (ERCP) technique, bile samples in patients without biliary diseases have been shown to be free from infection (10). However, any conclusions regarding the normal flora in human biliary tree also must await an extensive systematic bacteriological study.

1.2. Bacteriology of the biliary tract in biliary diseases

In contrast to the persistent ambiguities as to the bacteriology of the normal human biliary tree and liver, the frequent association of bile in-

fection with biliary diseases is agreed by many investigators. The frequency of infected bile obtained during percutaneous transhepatic cholangiography (PTC) or at operation for biliary disease ranges from 31 % to 80 %. These variations may be a reflection of the wide range in the type of biliary diseases studied (11-16). The incidence rates of infected bile in benign biliary diseases, such as acute cholecystitis, choledocholithiasis or benign bile duct stricture, are invariably high, i.e., 45-75 %, 58-83 % and 71-100 %, respectively (14, 15, 17-20). Chronic cholecystitis without stones usually gives lower figures (14, 21-23). On the other hand, in malignant biliary obstruction due to carcinoma of pancreas or ampulla, the incidence rate of bile infection reported in the past literature is characteristically low, ranging from 0 % to 16 % according to several investigations (14, 17, 18, 24). The exact mechanism(s) leading to divergent rates of bactibilia between malignant biliary obstruction and benign biliary disease has not yet been fully defined.

The concurrent presence of biliary lithiasis and malignancy has been shown in various ways (25, 26). Whether the presence or absence of a stone plays a decisive role in the infection rate of bile in malignant obstruction is still obscure. Recent works have revealed that the incidence of biliary tract infection in malignant obstruction often was underestimated in the past. Keighley (27) reported a rate of 36 %, McPherson et al. (28) of 32 % and Pitt et al. (15) of 46 %, figures which are by no means negligible.

The majority of organisms responsible for biliary sepsis are aerobic (strict aerobic or facultative anaerobic) bacteria. Aerobic bacteria in pure culture and mixed culture of aerobic and anaerobic bacteria comprise 87-100 % of the organisms isolated from the bile (9, 13, 24, 27). The most common pathogen is Escherichia coli (E. coli), which has been demonstrated to constitute 30-40 % of the isolates. Other frequently encountered facultative anaerobes are Streptococcus faecalis, Klebsiella aerogenes and Proteus species. These three organisms together with E. coli usually account for 75-85 % of the aerobic microflora (22, 24, 29). Although a pure culture of strictly anaerobic bacteria is unusual (9, 13), isolates of anaerobes from the bile range from 1 % to 39 % (16, 24, 30-32). In contrast to the rather consistent findings regarding the aerobic bacteriology in the bile in biliary disease, reports on anaerobic bacteriology of the bile have been somewhat confusing. Shimada et al. (9) and Roberts et al. (16) showed that anaerobes were found significantly more often in patients with common bile duct obstruction than those without ductal involvement. This correlation in the frequency of anaerobic infection with the ductal involvement was not detected by Lykkegaard Nielsen et al. (24).

The types of isolated anaerobes have been contradictory too. Clostridium (Cl.) perfringens was the most common strictly anaerobic isolate in the past (33-35). Recent improvement in anaerobic techniques has made it possible to isolate Bacteroides fragilis (B. fragilis). According to Lykkegaard Nielsen et al. (24), Shimada et al. (9), Bourgault et al. (13) and Roberts et al. (16), B. fragilis was a more common anaerobic pathogen than Cl. perfringens, whereas Keighley (27), McLeish et al. (36), Dye et al. (21) and Claesson et al. (20) could only rarely detect B. fragilis in the

bile. The discrepancy in the results of anaerobic isolation remains to be clarified.

The growth of B. fragilis has been known to be facilitated by the presence of bile (9). The poor recovery of B. fragilis by some investigators (20, 21, 27, 36), therefore, might be due to the use of inappropriate culture medium, such as thioglycollate broth, or due to a heavy growth of facultative anaerobes in the bile, which might have hindered the detection of B. fragilis. Dilution of the bile with a sensitive anaerobic technique may be useful for accurate determination of the isolates in the bile.

1.3. Risk factors for biliary infection

Infection of the bile in biliary disease is known to be frequent in elderly patients (7, 9, 14, 15). Other factors closely related to bactibilia are, previous attacks of cholecystitis (24), obstructive jaundice and common bile duct stones (21, 22). Keighley et al. (37) selected "high risk factors" by the prospective analysis of multiple preoperative and operative variables. Such risk factors for postoperative biliary infections are:

1. recent rigors,
2. emergency operation,
3. operation within 4 weeks of acute admission,
4. age over 70 years,
5. jaundice at operation,
6. previous biliary operation,
7. stones in the bile duct,
8. common bile duct obstruction.

Among these "high risk factors", factors 1.-3. seem to reflect the presence of bacteria in the bile under pressure. The reason why patients with factors 4.-8. are highly susceptible to bacterial infection of the bile has not been explained. The function of reticuloendothelial system (RES) might be the key to understand these findings. It is quite likely that aging is associated with a decline of reticuloendothelial (RE) function (38). A depression of RE function may also be provided by biliary operations causing a poor general condition due to malnutrition (39). The extent to which depression of RE function in biliary disease is related to the susceptibility for bacterial infection is yet to be defined.

1.4. Source and route of acquisition of bacteria in biliary infection

In the last two decades, interest has been focussed on the source and route of acquisition of bacteria in the bile in biliary diseases, and on factors that contribute to transforming "pathologic, but yet asymptomatic" bactibilia into "clinical cholangitis" (6).

The bacteria responsible for infection of the bile are almost always of the enteric type. Thus, an intestinal source of bacteria seems obvious, but whether bile infection is established by the translocation of bacteria from the intestine via the lymphatic path or hematogenous route, or simple ascent from the duodenum has been the matter of debate. Cameron

and Hou (40) suggested a hematogenous route of infection, stating that there is a continual bacterial traffic between the alimentary canal and the liver, and that the liver excretes microorganisms into the bile. The hypothesis led to the conclusion that all biliary obstructions would sooner or later be complicated by infection. However, a rather common finding that the incidence of bile infection in complete obstruction is lower than in partial obstruction (17, 18), speaks against the premises of Cameron and Hou (40). Furthermore, two prerequisites for the theory of a hematogenous route of infection are not yet established. One is the presence of portal bacteremia. In contrast to endotoxin, which is often demonstrated in portal blood (41, 42), portal bacteremia has previously been shown to be rare in man (43). However, studies on the translocation of intestinal microorganisms have suggested the portal entry of bacteria through the intestinal lymph (44), and an increase of portal anaerobic bacteremia by mesenteric ischemia (45). Whether enteric bacteria are more easily transferred to the portal vein in biliary disease than in the normal state should thus be clarified, although Freedman and Goldenberg (46) suggested that even a transient portal bacteremia of only a few hundred microorganisms might be sufficient to initiate hepatic infection. The other prerequisite for hematogenous infection is hepatic excretion of bacteria into the bile. Existing research has not solved the problem as to how the bacteria, taken up by the liver from portal blood, are excreted into the bile. However, this mechanism is supported by the observation of a lower frequency of bile infection in complete obstruction compared with partial obstruction. An increased intraductal pressure attained in complete obstruction could at last inhibit hepatocytic excretion of bacteria into the bile (47).

Since bacterial recovery from the gallbladder wall has been significantly more frequent compared to gallbladder bile (7, 24), Scott and Khan (18) have suggested that the organisms in the bile are derived from an infected gallbladder. Infection might be a consequence of bacteremia, however, the exact route by which bacteria find their way to the gallbladder and the significance of the special affinity of bacteria for the organ have not yet been explained.

Passage of bacteria from the intestine to the biliary tree through lymphatics may also be possible. The direction of lymph flow around the common bile duct is, however, towards the periduodenal and celiac group of lymph nodes and not towards the liver and gallbladder (48). Thus, the delivery of bacteria to the gallbladder by lymphatics might only be probable if a reversal of lymphatic flow has occurred.

An ascending route of infection is widely accepted, but still controversial. A premise for the establishment of an ascending cholangitis is the presence of enteric bacteria in the duodenum. It is well known that the stomach and the upper small bowel are sterile or contain very few organisms in the healthy human being. The microorganisms occasionally recovered consist mainly of gram-positive bacteria, such as streptococci, staphylococci and lactobacilli. They probably originate in the oral cavity from which they are carried down with feeding, and are able to survive the acidity of the stomach (49). Although coliforms are normally present in the upper small bowel of dogs, monkeys and rats (50), they are only occa-

sionally found in the upper small bowel in healthy human beings (51). Available data, however, have indicated that the normal microecology of the intestine is disturbed in biliary disease. Duodenal aspirates in biliary disease have been demonstrated to contain species of Enterobacteriaceae, which were identical with isolates from the bile recovered simultaneously (10, 52). But, the conditions which may alter the normal microecology of the intestine and prompt growth of coliform in the upper small bowel in biliary disease are largely unknown. Factors limiting bacterial growth in the upper small bowel are said to be (1) gastric acidity (53), (2) intestinal motility (54), (3) the ileocaecal valve (55), (4) mucus (56), (5) products of bacterial metabolism (57), (6) immunoglobulins (58) and (7) bile acids (59). Among these factors, gastric secretory activity has been the subject of frequent investigations in biliary disease, although the results are not consistent in all animals (60-63). A combination of achlorhydria (53) and biliary disease may provide a high frequency of bile infection. In general, bile acids inhibit gram-positive organisms, but have little effect on gram-negative organisms (59). Therefore, it is unexplained how the absence of bile acids in the intestine might play an active role in the establishment of Enterobacteriaceae colonization in the upper intestine.

In a condition of overgrowth of bacteria in the duodenum, any process which interrupts the normal function of the sphincter of Oddi may provoke biliary tract infection. The process of ascending bacterial infection might give a plausible explanation for the lower incidence of positive cultures in complete obstruction of the common duct than in partial obstruction. However, if one considers the development of biliary obstruction as a gradual process ultimately resulting in complete occlusion of the bile duct, contamination of the bile could occur similarly during the early periods of both diseases. Whether the frequency of bile infection is related to the rapidity of the occlusion process in complete biliary obstruction requires to be elucidated.

1.5. Mechanisms leading to biliary sepsis

The mere presence of bacteria in the biliary tree does not determine the clinical picture. The organisms could exist causing the pathological entity of cholangitis or inflammation of the bile ducts, without causing clinical cholangitis (6). The details of conditions responsible for converting asymptomatic bactibilia to frank biliary sepsis are still unknown. The mechanism obviously consists of a sequence of an increased intraductal pressure and regurgitation of bacteria into the systemic circulation. Huang et al. (64) demonstrated in dogs that bacteria began to appear in the blood at a bile duct pressure level above 250 mm H₂O. The bile duct pressure in acute obstructive suppurative cholangitis (AOSC), in which bacteria or endotoxin were detected in the blood, has been shown to be 300-460 mm H₂O (65). Normal pressures in healthy subjects have ranged between 70 and 140 mm of water (66). Thus, it is evident that a sudden increase in biliary pressure can cause bacteremia, provided the bile-blood barrier is damaged. Diagnostic procedures, such as PTC, operative cholangiography or T-tube cholangiography, are known to be able to precipitate cholangitis due to sudden overdilatation of the biliary tree and subsequent creation of an iatrogenic cholangio-venous

communication(s) (67, 68). In keeping with this mechanism, it has been suggested that the classic triad of Charcot (69, see below) is caused by an acute change in partial obstruction leading to a sudden increase in intraductal pressure (17). Flemma et al. (17) postulated that the increased pressure leads to rupture of the biliary mucosa. Also, since the bacteria injected into the bile duct passed over to the blood at pressures insignificantly exceeding the secretion pressure in dog, Jacobsson et al. (70) suggested that the bacterial transfer occurred through preexisting communications, which are closed when the pressure gradient from the biliary ducts to sinusoids and lymph spaces is normal.

The mechanisms of cholestasis and the route of regurgitation of bile constituents in extrahepatic biliary obstruction have been extensively studied for many years, although still conjectural. The bile canaliculi are separated from the intercellular space between liver cells only by the "tight" junctions. This intercellular space is continuous with the space of Disse, which communicates with the sinusoids via numerous pores (71). Thus, normally, tight junctions are recognized as the barrier which prevents passage of bile constituents from the bile canaliculi into the space of Disse. Using several particles, such as lanthanum, colloidal mercuric sulfide or horseradish peroxidase, which are likely to be larger than the size of any chemical compound naturally occurring in the bile, an increased permeability of the tight junctions in obstructive jaundice have been demonstrated (72-74) and conversely have been denied (75, 76). The route of regurgitation through the tight junctions has been designated as a paracellular pathway, whereas the investigators reporting contradictory findings suggested a transhepatocytic pathway for bile regurgitation. Bockman (77) stressed the necessity of "micropathology" of hepatic cells to allow escape of material into sinusoids by retrograde intrabiliary injection of ferritin. Whether even larger particles such as bacteria (0.5 μ -20 μ) could be transferred to the blood stream through such mechanisms has not yet been determined. Moreover, the movement of bacteria by their own motor activity should not be excluded a priori. However, the site and the mode of leakage from the bile may vary from time to time, during the course of the development of obstructive jaundice. It might be possible that the lumen of bile canaliculi has direct communications with the space of Disse. In the event of hepatocytic necrosis, bile canaliculi would certainly be destroyed since the cells undergoing degeneration contribute to the formation of the canalicular wall (78). Whether any of these events are closely associated with the pathology of patients being susceptible to clinical cholangitis awaits further investigation.

1.6. Clinical pictures of septic cholangitis

The typical signs and symptoms of acute obstructive cholangitis were first described by Charcot in 1877 (69). At that time the disease was characterized by complete obstruction of the common bile duct (CBD) with the subsequent accumulation of purulent material under pressure. This situation manifests as the diagnostic triad of Charcot which comprises jaundice, fever with rigors, and pain in the right upper quadrant of the abdomen. Reynolds and Dargan in 1959 (79) further characterized the syndrome with mental confusion or lethargy, and profound shock known as

Reynolds' pentad. These authors emphasized the needs for emergency decompression of the CBD. The first description of patients with gallstones presenting with such syndromes appeared in 1903 (80). The disease is not commonly encountered, the incidence being reported by Hauptert et al. as 1.7/1000 biliary diseases (81). but the mortality rate is invariably quite high (79, 82-84). The condition which fulfills the criteria of the pentad has later been designated as "acute obstructive suppurative cholangitis" (AOSC), a distinct clinical entity (82). Reynolds' pentad was seen in only 7 % of patients with suppurative cholangitis (81, 85). Incomplete Charcot's triad was found in 30-40 % in acute cholangitis with or without pus in the bile duct (1, 85). It has also been shown that septicemia is fairly common in non-suppurative disease (85). Thus, it is quite likely, as was suggested by Boey and Way (85), that the clinical toxicity represents a balance between, on the one hand, the virulence of the bacteria and the degree of purulent transformation of bile, and on the other hand, host resistance. Host resistance in acute cholangitis, however, has not yet been adequately investigated.

1.7. Treatment of biliary sepsis

1.7.1. Decompression procedures

The first attempts to drain the bile percutaneously from an obstructed biliary tree was reported in the 1960's (86-88). A subsequent successful report on the technique of percutaneous transhepatic biliary drainage (PTBD) by Molnar and Stockum in 1974 (89) has provided a new era in the treatment of obstructive jaundice. PTBD exhibited a dramatic effect in the moribund patients with acute suppurative cholangitis (90-92). Since the report of Mori et al. (93) and Nakayama et al. (91) that the mortality of surgery was reduced from more than 20 % to 7.6 and 8.2 %, respectively, PTBD has been widely accepted as a preoperative routine in the management of patients with biliary obstruction. However, the benefit of this technique has been challenged by the recent prospective studies. McPherson et al. (94) and Hatfield et al. (95) emphasized the disadvantage of an external drainage procedure because of its high complication rate. Among numerous complications including technical and mechanical ones, septic complications pose the most serious problem. This is due to the acquisition of environmental bacteria, such as Pseudomonas aeruginosa and Klebsiella aerogenes by the drainage system. Nilsson et al. (96) showed that infection of the bile was increased from the initial 30 % to 60 % in the jaundiced patients by the external drainage procedures. A closed drainage system devised by Blenkharn et al. (97) might be of help in preventing exogenous bacterial contamination and reducing septic sequences (98). However, as long as the catheter is kept in position, it carries the possibility of precipitating infection of the bile as it is a foreign matter. Furthermore, controlled clinical trials have failed to show an improvement of mortality by preoperative PTBD (95, 99, 100). The only objective improvement observed during drainage is a significant reduction in bilirubin. Nevertheless, it does not improve the mortality, indicating that the serum bilirubin level has nothing to do with the outcome of patients undergoing surgery. Current perioperative management which includes intravenous fluid load and antibiotic therapy might have alleviated the toxicity of bilirubin and endotoxin on renal function

(101-103) and increased the ability of patients to withstand major surgery.

Further disadvantages of external drainage system also is depletion of bile salts, fluids and electrolytes. In order to prevent this bile loss and the acquisition of exogenous bacteria, the usage of endoprosthesis to convert external drainage into internal drainage has been advocated (104, 105). The positioning of endoprosthesis by endoscopic method has also been developed (106). The internal drainage system seems attractive, however, the technique still has several problems. Endoprosthesis might become an obstacle to bile flow. It might act as a foreign material which precipitates infection. Clotting and dislodgement of endoprosthesis are known to occur. In cases of AOSC, it is quite unlikely that the thick pus in the bile ducts will be drained by either PTBD or an endoprosthesis system. Thus, further clinical studies and improvement of drainage systems are awaited.

1.7.2. Antibiotic therapy

Antibiotic cover is a cornerstone in the treatment of biliary sepsis. The efficacy of the antibiotic therapy depends on the sensitivity of biliary organisms to various agents, the concentration of these antibiotics in the bile, and the bactericidal activity of the bile. Factors that influence the extent of biliary secretion of pharmacological compounds include molecular weight, polarity and hepatic metabolism. However, biliary excretion of antibiotics studied so far has been poor, and the concentration of any agent has not reached the therapeutic level (i.e., 10 µg/ml), when the biliary tract was obstructed (107, 108). The strategy of antibiotic therapy during the last decade, therefore, has focussed on the prevention and reduction of postoperative morbidity, such as wound sepsis or septicemia in patients undergoing biliary surgery. Postoperative septic complications are more often encountered in patients with infected bile than in patients with sterile bile. Moreover, 64 % of wound infections and 90 % of episodes of septicemia were shown to be caused by organisms which had been previously isolated from the bile (27). Burke (109) and Stone et al. (110) emphasized the significance of prophylaxis. They demonstrated that postoperative initiation of antibiotics gave almost identical infection rates as if antibiotic had not been given. However, unnecessary prophylaxis has been condemned due to the risk of toxicity, hypersensitivity and the selective encouragement of resistant bacteria, and a danger of superinfection (111). It would seem that the current trend in antibiotic therapy in biliary surgery is directed towards the restriction of prophylactic antibiotics to high-risk patients (30, 35, 112, see 1.3.). In order not to give unnecessary antibiotic coverage, and not to delay the timing of administration, immediate gram staining during operation has been advocated (36, 113, 114). Accurate prediction of bile culture by this method has been 87 % (22, 113).

The ideal choice of antibiotics and the duration of therapy after surgical or non-surgical intervention are not yet clarified. It is likely that prolonged administration is warranted only after reconstructive biliary surgery, when the bile almost always contains microorganisms (17). In acute suppurative cholangitis, initial treatment is usually started

without knowledge of the responsible organism, therefore the antibiotics given must have a broad spectrum to cover all possible pathogenic organisms (115). Since less is known about the efficacy of different antibiotics for biliary infection and the proper way of administration, there may be a place for some other modality of treatment, for example, passive immunization with gammaglobulin, to replace antibiotic therapy in intractable biliary infection.

1.8. Host defense mechanism

1.8.1. Bacterial killing

Phagocytosis was first demonstrated by Metchnikoff in the 1880's (116) as a characteristic feature of the host defense mechanism against infection. It is generally believed that the neutrophils are almost entirely responsible for primary defense in acute localized lesions, whereas the reticuloendothelial system (RES) plays a major role in systemic infections such as septicemia (117). The process of neutrophil phagocytosis consists of adherence of bacteria to the cell by some recognition mechanism, incorporation of the organism into a phagocytic vacuole, phago-lysosome formation by fusion of lysosomal granule with the vacuolar membrane and the generation of a coordinated series of changes in oxidative metabolism termed the respiratory burst (118, 119). The respiratory burst includes the following changes (118): (1) increased consumption of O_2 , (2) increased flow of glucose through the hexose monophosphate shunt pathways, (3) increased production of superoxide (O_2^-) and hydrogen peroxide (H_2O_2), (4) increased fixation of inorganic iodide to a protein-bound form, (5) the generation of measurable light (chemiluminescence), and (6) increased reduction of the yellow dye nitroblue tetrazolium (NBT) to the black, insoluble product, formazan. Thus, the actual killing and digestion of microorganisms is accomplished through the action both of lysosomal granule contents (myeloperoxidase, acid hydrolases, neutral proteases, lysozyme etc.) (120) and of the oxidizing agents provided by the respiratory burst.

The mechanism of chemotaxis, phagocytosis and intracellular killing in macrophages seems to be the same as that in neutrophils. Due to their slow chemotaxis in comparison with neutrophil movement, mononuclear phagocytes (macrophages) play a minor role in the primary defense of localized infections.

1.8.2. The macrophage system

Aschoff (121) introduced the descriptive term "reticuloendothelial system (RES) in reference to a body of mesenchymal-derived cells which form a diffuse system of sessile and wandering mononuclear macrophages (122). The fixed macrophages localized in the blood sinuses of the liver, lung, spleen, and bone marrow participate to a major degree in the clearance of particulate matter from the blood. The macrophages in the liver and spleen manifest approximately 85-95 % of the total intravascular phagocytic activity (122).

More recently, the concept of a mononuclear phagocyte system (MPS) has a-

risen (123). The MPS includes monoblasts within the bone marrow, circulating peripheral blood monocytes, and the various tissue macrophages. These cells are related by common descent from a single precursor, the monoblast which originates in the bone marrow. The monoblast undergoes division and differentiation via promonocytes into monocytes, which are rapidly released into the circulation and randomly migrate into the tissues (124). Although the factors influencing monocyte localization in any particular tissue are poorly understood, approximately 50 % of circulating monocytes have been shown to take up residence ultimately in the liver sinusoids and differentiate into Kupffer cells (125). The functions of the MPS involve, (1) defense against microorganisms, (2) removal of dead or damaged cells, cell debris and inorganic material, (3) regulation of haematopoiesis, (4) cooperative and effector functions in the immune response, and (5) synthesis of biologically active compounds such as complement components, prostaglandins, interferon and neutral proteases (126).

1.8.3. Macrophage function

Reticuloendothelial system function has classically been measured by the colloid clearance techniques (127). Benacerraf et al. (128) have applied these techniques to a quantitative study of bacterial clearance. The kinetics of clearance of bacteria depend upon the homogeneity of the suspension, the efficiency with which the microorganisms could be phagocytized, and the dose injected. Observations of the fate of intravenously injected bacteria have shown that: (1) clearance of the bacteria followed an exponential function for the first few minutes, (2) the greatest proportion of bacteria (70-80 %) was taken up by the liver, (3) no difference could be detected between the clearance of live and heat-killed *E. coli* (128), (4) previous immunization of the animals increased the ability of the RES to extract bacteria from the blood (129), and (5) the rapid clearance of injected bacteria is followed, in the case of virulent organism, by a second phase when the blood concentration of the microorganism increases (130).

1.8.4. Opsonins

The efficiency of phagocytosis of bacteria has been extensively investigated in connection with "opsonins". Gram-negative bacteria such as *E. coli* are phagocytized poorly unless they are adequately opsonized by serum factors. Wardlaw and Howard (131) showed that staphylococci could be efficiently phagocytized by Kupffer cells of rat livers perfused with a bacterial suspension in Ringer's solution in the absence of serum, while removal of *E. coli* by the same liver preparation required both heat-stable and heat-labile components of serum. Opsonins, initially named by Wright and Douglas (132), are generally considered to include natural antibody, immune antibody, components of the complement system and substances such as fibronectin. The role of antibody and complement in opsonizing bacteria is mediated through affinity for IgG and C3. Opsonin manifests not only enhanced bacterial phagocytosis (133) but also helps removal of bacteria at a faster rate (134). Using a variety of foreign colloidal and particulate matter, RE depression after RE blockade (135) has been shown to coincide with opsonin depletion, and opsonin

activity is restored during recovery from RE blockade. Alexander et al. (136) has provided the evidence that components of the alternative complement pathway diminish during severe infection. They suggested that severe infection results in "consumptive opsoninopathy", which further increases susceptibility to bacterial infection. Lanser et al. (137) observed a depletion of opsonic fibronectin, a glycoprotein with a molecular weight of 440,000 (138), in patients with burn injuries within 12 hours of thermal injury, which was followed by restoration of normal levels at 24 to 48 h unless sepsis intervened; in the latter case a second prolonged depletion of opsonin occurred. Thus, prevention of RES failure by intravenous opsonic glycoprotein therapy has been tried after surgery, burn, trauma and sepsis and found to be of some benefit (139, 140).

1.8.5. The role of the spleen

The spleen plays a role in clearance of blood-borne particulate antigens, in cell-mediated immunity and in production of antibody and other opsonins (141-144). In the absence of specific antibody, the spleen constitutes the most effective clearance organ for bacteria (128, 145). Splenectomy therefore results in increased susceptibility to bacterial infection (146). Postsplenectomy sepsis is peculiar in that the mortality is more than 50 % (147). This overwhelming postsplenectomy infection in humans as well as animals is known to be caused mainly by encapsulated bacteria. Streptococcus pneumoniae is responsible for about 50 % of the septic events in splenectomized animals, whereas E. coli is reported to be responsible for 12 % of the postsplenectomy sepsis cases (147). Chaudry et al. (148) and Scher et al. (149) in their recent works emphasized the impaired capacity to clear such bacteria after splenectomy. Gullstrand et al. (150) using Streptococcus pneumoniae type 1, demonstrated that rats could develop overwhelming infection immediately after splenectomy. They suggested the importance of the nonspecific phagocytic system of the spleen for protection against pneumococcal challenge. Whether the finding is type specific to bacteria remains to be seen.

1.8.6. RE function in obstructive jaundice

RE depression has been suggested as a contributing factor to the frequent episodes of endotoxemia and renal failure occurring in obstructive jaundice (101, 103, 151, 152). However, relatively few studies have been reported in terms of measurement of RE function in obstructive jaundice. Following intravenous carbon particles injection, Halpern et al. (153) showed that biliary obstruction in rats increased the activity of the RES, which continued for about 5 weeks. Lásár (154) showed increased phagocytic activity 3 to 5 days after bile duct ligation in rats. In contrast, Holman and Rikkers (155) demonstrated that phagocytic index was significantly depressed at 21 days after biliary obstruction, although it was not different from control in animals at 3, 7, or 14 days after obstruction. Using iodinated human serum albumin (156), Drivas et al. (157) showed that at least half of a group of patients with obstructive jaundice had a defective Kupffer cell function. Wardle et al. (158) demonstrated that this depression of Kupffer cell function in obstructive jaundice occurs in parallel with impaired parenchymal cell function as measured by BSP. Katz et al. (159) demonstrated RES depression in ob-

structive jaundice in the rat using ^{35}S -labelled viable *E. coli*. The intravascular clearance of bacteria was not affected, however, hepatic phagocytosis was decreased and lung localization of bacteria was increased in biliary obstruction of 21 days. Although the significance of the lung localization of bacteria is still speculative, it is quite likely that the decreased phagocytic activity of the liver may contribute to the increased susceptibility to infection in chronic biliary obstruction.

1.9. Experimental biliary obstruction

Ligation of the CBD has been carried out as an experimental procedure of extrahepatic biliary obstruction for many years. Experimental models in the past have often been vitiated by the presence of infection, on one hand, and recanalization of the obstructed bile duct, on the other hand.

Infection was the invariable accompaniment to biliary obstruction in the literature up to 1900. These early findings are difficult to evaluate because of the poor operative and antiseptic techniques at that time. However, Cameron and Oakely (160) reported that infection with the formation of abscesses at the sites of necrosis of the liver was not uncommon in the rats after more than 11 days of biliary obstruction. A further increase of infectious complications has been noted in rats after more than 21 days' biliary obstruction (160, 161). Cameron and Hasan (162) suggested that infection was involved in the process leading to fibrosis, since fibrosis was slight when evidence of infection was meagre. On the other hand, "spontaneous" infection seems not to occur in animals with biliary obstruction of up to 7 days' duration (4, 48, 160), although the presence of indigenous bacterial flora in the liver and bile has been a continuous matter of debate. Thus, infections of the liver and bile may depend on the duration of biliary obstruction.

It was Brodie (163) who first observed that drainage of bile was restored 7 or 8 days after a single ligation of the bile duct in the cat. Since then the phenomenon of recanalization of bile duct after a simple ligation has been reported in several animals by several workers (78, 160, 164-167). Restoration of bile flow has occurred 14 days (78), 19 days (168) or 28 days (169) after bile duct ligation. Explanations for the recanalization have included: (1) slackening of the ligature at the site of obstruction (168), (2) prolapse of the walls of the dilated cranial part of the sac around the obstruction and subsequent adhesions between these and the caudal part of the duct followed by fusion (78), (3) new formation of liver lobules by proliferation of surviving liver cells and their linkage with redundant bile ducts (169), (4) the outgrowth of newly formed channels which arise from the biliary epithelium of the duct immediately caudal to the site of ligation and subsequent union with the distended cranial part (169). Trams and Symeonidis (78) showed that re-establishment of the bile flow was avoided by a small resection of the common bile duct.

1.10. Experimental models for biliary sepsis

Experiments to provide a biliary septic model have so far mainly been connected with the investigation of the route of infection in biliary ob-

struction. Freedman (170) demonstrated that intracardiac injection of bacteria led to bacterial recovery in the bile 3 h after injection, which persisted for 4 days in normal guinea-pigs. Dineen (48) showed in guinea-pigs with biliary obstruction of less than 4 days' duration, that the infection of bile was more prominent after intraportal inoculation of bacteria than after a systemic route of inoculation. Increased mortality has also been reported in cases with a combination of biliary obstruction and intraportal or intracaval injection of bacteria (161, 171). However, these previous studies have not been related with the different durations of biliary obstruction. Furthermore, with intravenous challenge of bacteria, the host might not be able to express various host defense mechanisms fully because of the sudden introduction of bacteria, and isolation of a septic focus might also be difficult (172). Since clinical cholangitis invariably results from a bacterial focus in the biliary tree, an animal model in which infection is originated in the biliary tree may be more useful in the study of biliary sepsis.

2. AIMS OF THE PRESENT STUDY

There are persisting demands for establishing animal models of biliary sepsis for the investigation of the mechanism(s) leading to sepsis in biliary obstruction. In order to provide such an experimental model, a method of retrograde intrabiliary (RI) injection of bacteria which simulates the ascending invasion of bacteria from the duodenum was developed in rats with biliary obstruction (I).

In order to study the relation between susceptibility to bacteria and the duration of the biliary obstruction, RI challenge with a standardized E. coli preparation was performed in rats with biliary obstruction of different durations (II).

In order to clarify the relative contributions of organs other than the liver in the protection against biliary sepsis, the effect of splenectomy on the susceptibility to RI challenge of bacteria was studied in otherwise normal rats (V).

The occurrence of frequent biliary infections in obstructive jaundice suggests an impaired capacity of the individuals with chronic biliary obstruction to cope with the ascending bacteria. Bacterial clearance from the biliary tree was therefore studied in an obstructive jaundice model (III).

As the liver plays a major role in the local defense in biliary sepsis, the bacterial clearance capacity within the liver was evaluated after incubation of bacteria in the biliary tree for different periods of time (IV).

It is probable that if immune defense mechanism were enhanced then protection against bacterial infection would be improved. The effect of immunization on blood clearance and organ localization of bacteria was therefore studied in rats with chronic biliary obstruction (VI).

RE function in rats with biliary obstruction was also evaluated by measuring the colloidal uptake rate using scintillation camera technique (VII).

3. MATERIALS AND METHODS

3.1. Animals

Inbred albino Sprague-Dawley rats were used in all experiments. The animals were maintained on an artificial day and night cycle and fed laboratory food (pellets) and water *ad libitum*. Fifty rats of both sexes, weighing 220-340 g were used for the experiments in paper I, and 269 male rats, weighing 250-370 g were used in papers II-VII.

3.2. Bacteria

A randomly selected Escherichia coli (E. coli) strain was isolated at our routine bacteriological laboratory from a wound, unrelated to biliary sepsis. The strain was cultured in 10 ml Todd Hewitt broth overnight at 37 °C. After centrifugation at 3000 g for 15 min, the pellet was suspended in serum broth to an extinction of 0.70 in a Linson 3 photometer, measured against fresh serum broth as blank. After incubation for 90 minutes in a 37 °C water bath, the bacterial suspensions were divided into 1 ml aliquots, quickly frozen using liquid nitrogen and stored at -80 °C, as previously described for pneumococci (173). Immediately before use, the bacteria were thawed and diluted in Todd Hewitt broth to obtain 0.1 ml volumes containing 10^8 (paper I), 10^5 (I, II, V, VI) or 10^2 (I) colony forming units (CFU), respectively.

3.3. Radiolabelling of bacteria

The E. coli strain was cultured overnight in 10 ml Todd Hewitt broth, heat-killed at 80 °C for 30 min and washed three times in phosphate-buffered saline (PBS, 0.03 M phosphate, 0.12 M NaCl, pH 7.2) followed by suspension in 4 ml PBS. The bacteria were then labelled with ^{125}I according to the protein-labelling method of MacConahey and Dixon (174), using chloramine-T as an oxidizing agent. After three further washings in PBS, the bacteria were frozen in aliquots at -20 °C and used within 2 weeks. The radioactivity was approximately 2×10^6 counts per minute (cpm) (III, IV) or 3×10^6 (VI) cpm for 2.5×10^8 /ml bacterial suspension.

3.4. Test substance in scintillation camera technique

The test substance, $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid ($^{99}\text{Tc}^{\text{m}}\text{S}_7$) was prepared according to Persson and Naversten (175), using 48 μmol sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$) and 9 μmol potassium perrhenate (KReO_4). The particle size of the colloid estimated by scanning electron microscopy ranged from 200 nm to 500 nm with a mean of 300 nm (176). The number of particles per ml of solution was approximately 10^{10} . The labelling efficiency tested with gel chromatography column scanning technique (177) was 97-100 % (VII).

3.5. Bacterial vaccine

The vaccine was prepared from an overnight culture of the E. coli strain in Todd Hewitt broth at 37 °C. After incubation, the bacteria were sedimented by centrifugation, washed twice in sterile saline, and resuspended in saline followed by heating at 80 °C for 30 min. After control of the

sterility, the bacterial concentration was adjusted spectrophotometrically to an optical density of 1.0 at 540 nm, corresponding to approximately 10^{10} E. coli per ml. The vaccine was used for experiments in paper VI.

3.6. Quantitation of rat IgG antibodies to surface antigens of E. coli

Serum for determination of antibody was taken from rats with biliary obstruction before and 2 weeks after vaccination (VI). Staphylococcal protein A, which reacts with the Fc-fragment of rat IgG1 and IgG2c (178) was radiolabelled with ^{125}I (174). The E. coli strain previously used for the immunization was grown overnight in Todd Hewitt broth at 37 °C, washed three times in PBS and suspended in 1/10 volume PBS. To 200 μl E. coli suspension was added 200 μl PBS containing 0.1 % Tween 20 (Kebo-Grave, Stockholm, Sweden) and 100 μl test serum. After incubation at 37 °C for 30 min, 2 ml PBS-Tween was added, the tube was centrifuged at 3000 g for 15 min, and the pellet suspended in 200 μl PBS-Tween. Radiolabelled protein A, 50 μl (0.2 μg) was added, followed by another 2 ml PBS-Tween and centrifugation and washed by 0.1 % PBS. The radioactivity in the washed pellet was then counted and the results expressed in counts per minute (cpm) as the mean of duplicates. The background activity for the test, as measured with PBS instead of rat serum was approximately 300 cpm.

3.7. Anaesthesia and surgical procedures

All operations including injections of bacteria and measurement of RE function (see below) were performed under ether anaesthesia using clean but not sterile conditions.

3.8. Biliary obstruction

Extraheptic obstruction of the common bile duct (CBD) was created by double ligation and excision of 5-8 mm of the CBD between the lowermost tributary of the bile duct and the uppermost tributary of the pancreatic duct (I-VII) according to Trams and Symeonidis (78). Sham operation consisted of isolation of the CBD without ligation (VI, VII).

3.9. Splenectomy

Splenectomy was performed via a midline laparotomy after mobilization of the spleen and individual ligation of the splenic vessels (V). Sham operation included splenic mobilization and resection of a small part of the greater omentum after individual ligation of the omental vessels (V) (150).

3.10. Method of retrograde intrabiliary (RI) injection of bacteria

For bacterial challenge in the normal rats, the CBD was isolated and ligated (6-0 silk) just above the entrance of the highest branch of the pancreatic duct. Immediately after ligation, RI injection of 0.1 ml of E. coli was performed proximally above the ligature with a fine hypodermic needle (0.2 mm diameter). The inserted needle was tied in to the bile duct by another thread, in order to minimize leakage of bacteria into the

intraperitoneal space, and the injection was performed very slowly (over at least 40 seconds) with minimal pressure (I, II, V). After withdrawal of the needle, the proximal CBD just above the needle hole was ligated. In almost all cases, a part of the CBD between the ligatures was resected. When making a RI injection of bacteria in the CBD of an obstructed rat, a purse-string suture (6-0 silk) was applied to the dilated CBD to prevent leakage of the bacteria (I, II, V, VI).

For the study of bile clearance of bacteria (III, IV), the CBD was cannulated with a silastic catheter (Dow Corning Corporation, Michigan, USA; 0.012 in. ID, 0.025 in. OD). RI injection of ^{125}I -labelled *E. coli* was performed via this cannula with a slow minimal manual pressure (III), or by using an infusion pump (Harvard Apparatus Company, Milis, Mass., USA; Model 940) at a rate of 32.8 $\mu\text{l}/\text{m}$ (IV).

3.11. Bacterial challenge

RI injection of *E. coli* was performed in 46 normal rats (I, II, V), 77 rats with biliary obstruction of different durations (I, II, V, VI) and 10 sham operated rats (VI). Intraperitoneal (IP) injection of bacteria was performed in 8 normal rats and 6 jaundiced rats (I). Intravenous (IV) injection of bacteria was performed in normal rats (12), rats with splenectomy (12) and rats with simultaneous obstruction of the CBD and splenectomy (12) (V). For IV injection of bacteria, the lateral vein of a hind limb or the penile vein was used.

After challenge, the animals were kept under standard laboratory conditions with food (pellets) and water ad libitum and observed every sixth hour for 7 days. An autopsy was performed when rats were dead and 0.1 ml heart blood and bile were spread on blood agar plates. After overnight incubation at 37 °C, randomly selected colonies were identified using a routine fermentation reaction. Surviving rats were sacrificed 7 days after challenge and samples from heart blood and bile were taken for culture.

3.12. Measurement of bacterial clearance and organ distribution

Bile clearance of bacteria was measured after RI injection of ^{125}I -*E. coli* (III). The bile cannula was unclamped immediately after injection, and the bile was allowed to drain freely. Ten μl samples were taken at 1 min interval until 15 min and then at 10 min interval until 120 min. The rate of bile flow was also measured. The 10 μl samples were used for the measurement of radioactivity in the bile during each time interval.

Blood clearance of bacteria was measured after IV injection of 0.1 ml of ^{125}I -*E. coli* via a jugular vein (VI). 0.5 ml of blood was withdrawn from the femoral vein at 1, 3, 5, 7 and 10 min. Bacterial clearance rate (K) was determined using the expression of $K = 0.301/T_{1/2} (1)$ where $0.301 = \log_{10} 2$ and $T_{1/2}$ is the intravascular half-time (122).

Organ distribution of bacteria was determined immediately after measurement of bacterial clearance from the bile (IV) or from the blood (VI). After exsanguination by aortic puncture, the liver, followed by excision

of the extrahepatic bile duct, spleen, lungs and kidneys were removed and weighed. The bile collected during the first 15 minutes of drainage was pooled together with bile subsequently aspirated from the distended bile duct (IV). The radioactivity of the samples was determined using a 1260 Multigamma counter (LKB, Stockholm, Sweden), with a background not exceeding 60 cpm. The radioactivity of the bile was summed with that of the extrahepatic bile duct and expressed in per cent of the total amount of ^{125}I -labelled E. coli injected into the rat. The distribution of bacteria in the different organs was expressed in per cent of the injected bacteria, per gram of tissue (VI) or in the total organ (IV, VI). In the paper (IV), in order to investigate the capacity of liver clearance for E. coli, bile was collected during 15 min, immediately, 1 h, 4 h or 24 h after RI injection. In the paper (VI), the blood clearance and organ localization of bacteria was determined by IV injection of ^{125}I -labelled E. coli in rats with or without prior immunization.

3.13. Measurement of RE function by scintillation camera technique and computer analyses

After positioning ether anaesthetized animals on a scintillation camera (Maxi Camera, General Electric) equipped with a Lehr parallel hole collimator, about 15 MBq in a volume of 0.5 ml test colloid was injected rapidly, during 2-4 seconds, into the superior vena cava via the jugular vein (VII). Sequential images were taken during 15 min from the start of injection. Three frame groups were stored with 16, 12 and 20 frames at intervals of 7.5, 15 and 30 seconds, respectively. Regions of interest were selected in the image and time activity curves were generated. The curves were stored and transferred to a computer (Digital Equipment PDP 11/45) where the uptake curves could be later evaluated. To estimate the flow rates of the radiolabelled colloid through the liver (K_1) and through other RE tissues (K_2), an open two-compartment model was assumed (179). The clearance of the colloid from the blood (B) and the rate of uptake in the liver (L) can be written:

$$\frac{dB}{dt} = -B (K_1 + K_2) \quad (2)$$

$$\frac{dL}{dt} = BK_1 \quad (3)$$

The amount of colloid transported to the liver could thus be expressed by the equation:

$$L(t) = 100 \frac{K_1}{K_1 + K_2} \{ 1 - e^{-(K_1 + K_2)t} \} \quad (4)$$

By fitting this equation to the experimental curves for each animal, the rate constants K_1 and K_2 were calculated. According to the calculation of the corrected phagocytic index proposed by Biozzi et al (127), the corrected colloidal uptake rate (cK_1) of the liver was thus calculated:

$$cK_1 = \sqrt[3]{K_1} \cdot \frac{W}{WL} \quad (5)$$

where W represents the body weight, and WL represents the weight of the liver.

After completion of the registration of the scintillation camera, all animals were exsanguinated by aortic puncture. After the liver, the lungs and the spleen were resected and bone marrow was aspirated from both femurs, all these tissues were inserted into plastic tubes, weighed and measured for radioactivity with an automatic sample-changing NaI (Tl) well counter.

3.14. Statistical analysis

Survival rates were evaluated by Fisher's exact (probability) test (I, II, V, VI). Differences in bile radioactivity (III) and survival time (V) were evaluated using Mann-Whitney's rank sum test. Organ distribution of radiolabelled bacteria was compared by Student's t test (IV) or Wilcoxon's test (VI). Studies in the paper (VII) were exclusively evaluated by Student's t test.

4. RESULTS

4.1. Effect of CBD obstruction in rats

Ligation and excision of a segment of the CBD in rats resulted in dilatation of the proximal CBD and enlargement of the liver. The increase in diameter of the CBD was proportional to the duration of the obstruction. Those rats with more than 3 weeks' biliary obstruction invariably showed a pronounced cystic dilatation of the bile duct. Derangements of liver function tests were noted as early as 1 day after biliary obstruction. These abnormal biochemical data persisted for several weeks (Table I, Tanaka unpublished data). Clinical jaundice was obvious from discolora-

Table I. Liver function tests in rats with biliary obstruction^a

Duration of obstruction	No. of rats	Bilirubin (3-20 μ mol/l)	ALP (0.8-4.6 μ kat/l)	GT (< 1.0 μ kat/l)	ASAT (< 0.67 μ kat/l)	ALAT (< 0.67 μ kat/l)
1 day	5	46.2 $\pm$ 1.3	35.2 $\pm$ 3.0	0.1 $\pm$ 0.0	9.3 $\pm$ 1.2	7.3 $\pm$ 0.4
3 days	6	93.0 $\pm$ 5.0	20.1 $\pm$ 1.5	0.2 $\pm$ 0.1	5.5 $\pm$ 1.8	5.0 $\pm$ 1.7
1 week	5	77.6 $\pm$ 4.0	17.7 $\pm$ 0.5	0.2 $\pm$ 0.1	3.4 $\pm$ 0.3	1.6 $\pm$ 0.1
2 weeks	6	102.1 $\pm$ 9.8	22.0 $\pm$ 1.0	0.3 $\pm$ 0.0	5.4 $\pm$ 1.1	1.9 $\pm$ 0.2
3 weeks	9	120.6 $\pm$ 6.2	24.2 $\pm$ 2.1	1.2 $\pm$ 0.3	3.6 $\pm$ 0.4	1.6 $\pm$ 0.3

^aData are expressed in mean $\pm$ SEM.

tion of skin, eye and urine. The body weights decreased until the third day of biliary obstruction, but increased steadily thereafter exceeding the initial weight at 7th day, although the weight gain was less compared with that in sham-operated rats (Fig. 1, Tanaka unpublished data). The organ weights increased after 1 week's biliary obstruction compared with sham operation ($p < 0.005$, t-test) (VII). A further increase of the relative weight of the liver and spleen was noted after 3 weeks' obstruction compared to 1 week's obstruction ($p < 0.005$, t-test) (Table II).

4.2. Effect of RI injection of E. coli in rats with CBD obstruction

RI injection of bacteria in rats with biliary obstruction caused general signs of illness in all animals immediately after injection. They showed tachypnoea, piloerection, low physical activity, and reduced intake of food and water (I, II). Consequently, the body weights decreased in septic rats. These signs were generally not found in control rats following RI injection of bacteria. Laboratory findings 12 h after bacterial challenge in rats with 3 weeks' biliary obstruction showed a typical "septic" course with decreases in platelets and leukocytes and an increase of the

hematocrit (Ht), without significant changes in liver function, activated partial thromboplastin time (APT-time), or hemoglobin (Hb) (Fig. 2, Tanaka unpublished data).

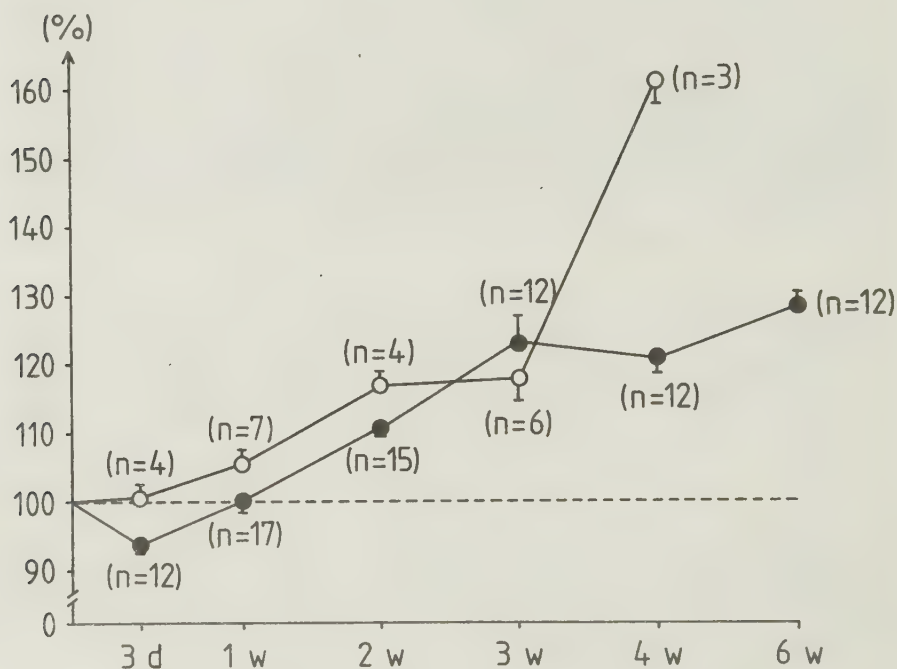


Fig. 1. Body weight changes in rats with sham operation (open circle) and rats with biliary obstruction (closed circle).

Table II. Relative weights (percentage of total body weight) in liver and spleen in rats with biliary obstruction and sham-operation^a.

Groups	No. of rats	Body weight	Liver weight	Liver (%)	Spleen weight	Spleen (%)
A. Sham operation	7	317±23	12.1±0.8	3.82±0.25	0.8±0.1	0.26±0.03
B. 1 week obstruction	8	284±12	15.2±2.1	5.34±0.72 ^b	1.0±0.2	0.36±0.06 ^b
C. 3 week obstruction	8	340±24	22.9±3.2	6.71±0.70 ^c	1.7±0.3	0.49±0.09 ^c

^aData are expressed as mean ± SD

^bp < 0.005 compared to sham operation

^cp < 0.005 compared to both sham operation and 1 week biliary obstruction

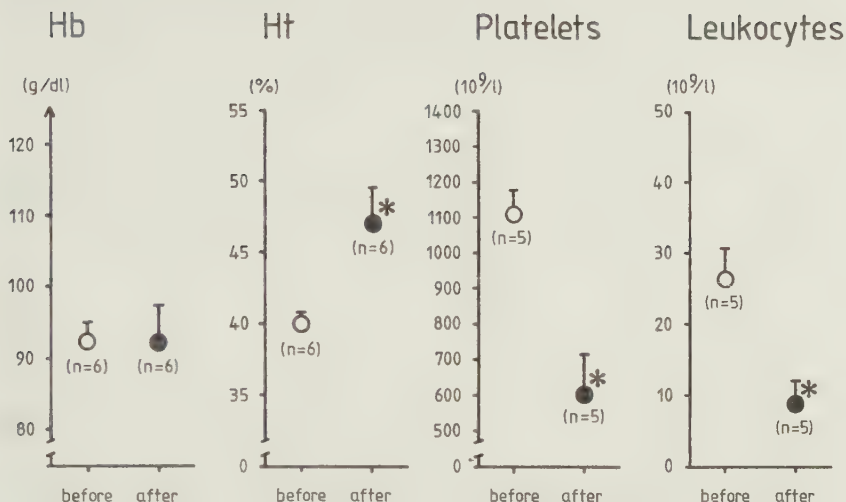


Fig. 2. Hematological study in rats with 3 weeks' biliary obstruction before (open circle) and 12 hours after (closed circle) bacterial challenge with 10^5 E. coli. * $p < 0.05$.

The outcome of bacterial challenge with different doses of E. coli in control rats and rats with 3 weeks' biliary obstruction is shown in Table III (I). Control rats withstood RI challenge with 10^8 E. coli,

Table III. Outcome of bacterial challenge of normal rats and animals with chronic biliary obstruction

Groups of animals	Challenge dose of <u>E. coli</u>	Route of challenge	Number of animals	Number of dead animals
Control rats	10^2	RI ¹	8	0
	10^5	RI	8	0
	10^8	RI	8	0
	10^5	IP ²	8	0
Rats with 21 days' chronic biliary obstruction	10^2	RI	8	5*
	10^5	RI	4	4**
	10^5	IP	6	0

¹RI: retrograde intrabiliary injection.

²IP: intraperitoneal injection.

* Significantly more animals died in this group as compared to each group of normal rats ($p = 0.0128$) or the IP injected animals ($p = 0.0300$).

** $p < 0.005$ compared to normal rats.

whereas 62 % of rats with chronic biliary obstruction died from 10^2 E. coli and all such rats died from 10^5 E. coli. The results of the susceptibility to RI challenge of 10^5 E. coli in rats with biliary obstruction of several durations are summarized in Table IV (I, II, V, VI). After 3

Table IV. Summary of outcome in RI challenge of 10^5 CFU E. coli

Groups of animals	Paper	Number of animals	Number of dead animals	Mortality (%)	p-value ^a
1. Control (sham)	I, II, V, VI	48	4	8.3	-
2. 3 days' jaundice	II	11	6	55	0.001
3. 2 weeks' jaundice	II	12	7	58	0.004
4. 3 weeks' jaundice	I, V, VI	20	20	100	< 0.000001
5. 4 weeks' jaundice	II	12	10	83	< 0.000001
6. 6 weeks' jaundice	II	12	10	83	< 0.000001

^a p-value was calculated by comparison with normal rats using Fisher's probability test. When groups 2 and 3 were combined and compared with groups 4-6, the difference was significant ($p < 0.002$).

days of biliary obstruction the susceptibility to bacterial infection was already increased. When the two early groups of animals (3 days' and 2 weeks' jaundice) were combined and compared to the animals with more longstanding jaundice (3, 4 and 6 weeks), a significant difference ($p < 0.002$, Fisher's test) in the susceptibility to E. coli was observed.

Modification of the susceptibility to bacterial RI challenge by splenectomy (V) or active immunization (VI) was also investigated. The mortality rate in control rats with intact spleens was only 8.3 % after RI challenge. RI injection of the same dose of bacteria immediately after splenectomy resulted in 75 % mortality (Table V). On the other hand, immunization against E. coli (see below) could not modify the outcome of RI bacterial challenge in rats with chronic biliary obstruction (Table VI).

Almost all rats dying with biliary obstruction died within 3 days after RI challenge. The median survival time of dead animals was 23 h (range 18-40 h) after 3 days' obstruction (II), 23 h (19-56 h) after 2 weeks' jaundice (II), 18 h (16-21 h) after 3 weeks' jaundice (V), 40 h (20-84 h) after 4 weeks' jaundice, and 44 h (12-56 h) after 6 weeks' obstruction (II), respectively. In contrast, the median survival time of dead animals in RI-injected, splenectomized rats without previous biliary obstruction (V) was 125 h (range 18-158 h), which was significantly longer ($p < 0.05$, Mann-Whitney's test) than the survival time in chronically jaundiced rats.

Bile cultures obtained from the biliary obstructed animals immediately before bacterial challenge were negative (two rats with infected bile (I) were excluded from the subsequent study). Both blood and bile cultures from all dead rats after RI challenge grew E. coli (I, II, V, VI). Blood cultures from the surviving animals at sacrifice at 7th day were

Table V. Outcome of the challenge with 10^5 colony forming units (CFU) E. coli

Group	CBD obstruction ^a	Splenectomy	Route of challenge ^b	No. of dead rats/ No. of challenged rats
I	+	+	RI	9/12 ^c
II	+	-	RI	1/12
III	+	+	IV	0/12
IV	-	+	IV	0/12
V	-	-	IV	0/12

^aCBD (common bile duct) obstruction in groups I, II and III was performed in connection with bacterial challenge.

^bRI: retrograde intrabiliary injection. IV: intravenous injection.

^c_p < 0.005 compared to groups II-V.

Table VI. The susceptibility of different groups of rats to retrograde intrabiliary (RI) injection of E. coli^a

Groups	Vaccination against <u>E. coli</u>	No. of challenged rats	No. of killed rats
Sham-operation	No	10	0
Obstruction of the common bile duct	No	10	10 ^b
Obstruction of the common bile duct	Yes	10	9 ^b

^aA dose of 10^5 CFU was given.

^b_p < 0.0001 compared to sham operated rats.

- all negative except one normal rat (I). However, almost all bile cultures from surviving jaundiced rats (I, II), and approximately half of the bile cultures from the surviving normal (I, II, V) or sham-operated (VI) rats were positive for E. coli.

4.3. Intraperitoneal (IP) and intravenous (IV) injection of E. coli

In contrast to the susceptibility to RI injection of 10^5 E. coli in rats with jaundice or in splenectomized rats, IP injection of the same dose of

bacteria did not kill any of the normal or the jaundiced rats (I) (Table III). IV injection did not cause any deaths in normal rats with simultaneous ligation of the CBD and splenectomy (V) (Table V).

4.4. Bacterial clearance from the biliary tree

In order to study the mechanism behind the susceptibility to RI injection of bacteria in rats with biliary obstruction, bacterial clearance from the biliary tract was studied (III, IV). RI injection of radiolabelled *E. coli* and subsequent collection of the bile in control and chronically obstructed rats gave a characteristic clearance pattern from the bile as shown in Fig. 3 (III). The decline of radioactivity recovered in the

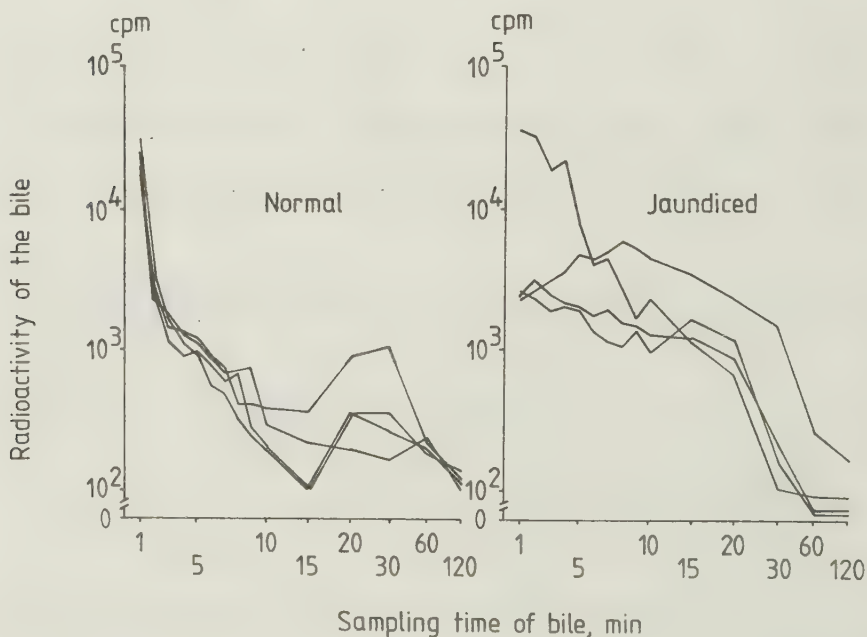


Fig. 3. Clearance of ^{125}I -labelled heat-killed *E. coli* from the bile in 4 normal rats (left) and 4 rats with 3 weeks' obstruction of the common bile duct (right). The *E. coli* preparation, 0.1 ml, containing 2×10^5 counts per minute (cpm) was injected in the biliary tree and samples of 10 μl were collected at times indicated on the abscissa. Ordinate: radioactivity in cpm in the bile samples (log scale)

bile from 1 min to 2 min was steep in control rats. The radioactivity after 2 min declined exponentially for the following 10 min in normal rats. Bile samples collected from 2 min to 15 min after injection showed higher amounts of radioactivity in rats with biliary obstruction than in

control rats ($p < 0.05$). When the radioactivity excreted during the first 15 min by the two groups of animals was estimated by the calculation of the area "below the curves" in Fig. 3, the obstructed rats excreted more *E. coli* than the normal rats. The clearance rate of bacteria was estimated by the formula:

$$K = \frac{(\log C_5 - \log C_{10})}{T_{10} - T_5} \quad (6)$$

where C_5 and C_{10} represented the radioactivity of the bile at the time T_5 (5 min) and T_{10} (10 min), respectively. A significantly higher clearance rate was found in the control rats; mean $K = 0.1158$ (range 0.1394-0.0869), compared with 0.0473 (range 0.0793-0.0061) in the obstructed rats ($p < 0.05$, Mann-Whitney's test).

4.5 Bacterial clearance capacity of the liver

After incubation of ^{125}I -labelled *E. coli* in the CBD for different periods of time, the liver clearance capacity for bacteria was measured (IV). In normal rats, 40 % of the bacteria were recovered in the bile during the 15 min collection period immediately following the injection of bacteria, whereas 30 % were already trapped in the liver (Fig. 4). One hour incuba-

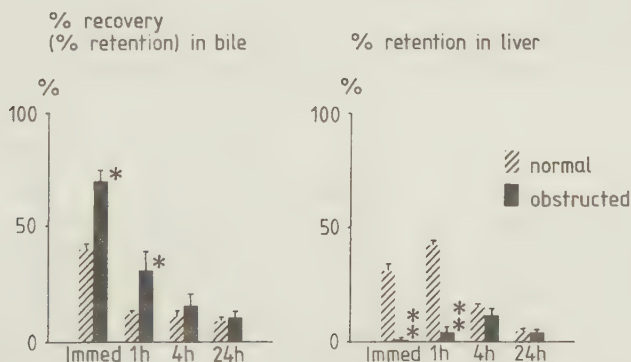


Fig. 4. Recovery of the radioactivity in the bile/biliary tree (left) and retention in the liver (right) in normal rats (hatched bar) and rats with chronic biliary obstruction (closed bar) after retrograde intrabiliary injection of ^{125}I -labelled *E. coli*. Abscissa: incubation period (Immed: immediately after injection, 1 h, 4 h and 24 h) after retrograde intrabiliary injection. Ordinate: radioactivity in per cent of the injected dose of bacteria. Each mean value was obtained from 8 control rats or 5 obstructed rats. Bars indicate SEM. * $p < 0.05$ compared to control rats. ** $p < 0.005$ compared to normal rats. When compared with Immed value, recovery in the bile was significantly decreased ($p < 0.01$) at 1 h, 4 h and 24 h, respectively, in both groups of animals. Retention in the liver was significantly increased ($p < 0.01$) at 1 h, but decreased ($p < 0.01$) at 4 h and 24 h in normal rats. Retention in liver was significantly increased ($p < 0.01$) at 4 h in obstructed rats. (t-test)

tion of bacteria in the CBD in control rats reduced the recovery of bacteria in the bile to 13 % ($p < 0.01$, t-test) and increased the retention in the liver to 43 % ($p < 0.01$, t-test). Further prolongation of the incubation period resulted in a slight decrease in radioactivity in the bile, but the retention in the liver decreased gradually to 15 % ($p < 0.01$) after 4 h and to 4 % ($p < 0.01$) after 24 h incubation (Fig. 4).

The initial trapping of bacteria in the liver in the rats with chronic biliary obstruction was significantly impaired compared to control rats. More than 70 % of the injected bacteria was retained in the biliary tree 15 min after injection and only 1 % was trapped in the liver. One hour incubation of bacteria in the CBD increased the retention in the liver, but it was still significantly less than in control rats. Four and 24 h after RI injection of bacteria, however, less than 30 % was retained in the hepatobiliary system in both groups of animals (Fig. 4). However, most of the animals showed almost no radioactivity exceeding the background count in the blood, urine, spleen, lungs and kidneys even after a prolonged incubation period.

4.6. Vascular clearance of ^{125}I -labelled *E. coli*

Intravascular clearance capacity of ^{125}I -labelled *E. coli* and effect of immunization was studied (VI). Quantitation of IgG antibodies to surface antigens of *E. coli* strain used for RI challenge demonstrated that there was a more than 20-fold increase in antibodies after immunization with the homologous strain (Fig. 5). Blood clearance studies showed that there

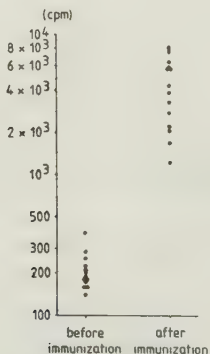


Fig. 5. Quantitation of IgG antibodies to surface antigen of *E. coli* before and after immunization with heat-killed *E. coli* vaccine in rats with chronic biliary obstruction. Quantitation of antibodies was expressed as radioactivity in counts per minute (cpm). (Ordinate; log scale.)

were no significant differences in the clearance rate for ^{125}I -labelled *E. coli* between sham-operated rats and rats with chronic biliary obstruction: K-value in non-immunized and immunized sham operation, and non-immunized and immunized biliary obstruction was 0.18 ± 0.02 , 0.16 ± 0.01 , 0.17 ± 0.02 , and 0.18 ± 0.01 , respectively.

A localization of the bacteria to the liver and spleen expressed as a percentage of injected bacteria was significantly decreased in chronically obstructed rats ($p < 0.005$, Wilcoxon's test) compared to sham operated rats (VI) (Table VII). When immunized and non-immunized animals with

Table VII. Organ localization per unit weight of intravenously injected ^{125}I -labeled *E. coli* in different groups of rats^a

Groups	Vaccination against <i>E. coli</i>	Liver	Spleen	Lungs	Kidneys
Sham-operation	No	2.98 ± 0.24	3.51 ± 0.54	0.27 ± 0.02	0.966 ± 0.005
Sham-operation	Yes	3.87 ± 0.08	1.81 ± 0.19^a	0.38 ± 0.06	0.073 ± 0.004
3 weeks' obstruction of the common bile duct	No	1.68 ± 0.69^b	0.83 ± 0.15^b	0.39 ± 0.03^a	0.067 ± 0.006
3 weeks' obstruction of the common bile duct	Yes	1.92 ± 0.18^b	0.86 ± 0.15^b	1.47 ± 0.32^c	0.106 ± 0.010^c

^a Values represent mean $\pm$ SEM (n = 6 in each group) expressed as per cent of injected bacteria per gm of tissue.

^a $p < 0.05$ compared to non-immunized sham-operated rats. ^b $p < 0.05$ compared to both sham operated groups. ^c $p < 0.005$ compared to all other groups.

CBD obstruction were compared, the immunized rats demonstrated a significantly higher bacterial trapping in the lungs and kidneys per gram basis ($p < 0.005$, Wilcoxon's test). Studies on organ localization of bacteria in relation to total organ mass (Fig. 6) showed that there were no differences between the four groups of animals investigated with respect to liver uptake of bacteria (VI). However, the uptake in the lungs of the animals with chronic biliary obstruction was significantly increased, as compared to sham operated animals. Furthermore, the immunized rats with chronic biliary obstruction showed a significantly higher uptake of the lung than the corresponding non-immunized animals (Fig. 6). The immunized rats with chronic biliary obstruction showed lower uptake of bacteria in the spleen and higher in the kidneys than the sham operated animals.

4.7. RE function measured by $^{99}\text{Tc}^m$ -sulphur colloid

RE function was evaluated by measuring the biokinetics of a standardized $^{99}\text{Tc}^m$ -sulphur colloid in rats with biliary obstruction (VII). The uptake rate of $^{99}\text{Tc}^m$ -sulphur colloid in the liver (K_1) was not different between the rats with biliary obstruction and those with sham operation. However, the uptake rate of the extrahepatic RES (K_2) was significantly higher ($p < 0.005$, t-test) after 3 weeks' biliary obstruction than after sham

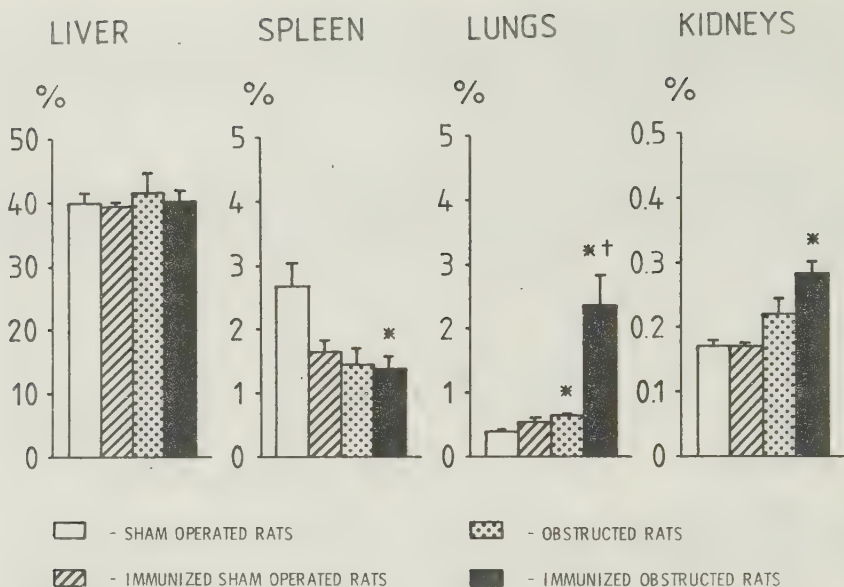


Fig. 6. Organ distribution of intravenously injected ^{125}I -labelled *E. coli* in experimental groups. Values represent mean $\pm$ SEM expressed as per cent of injected bacteria per total organ. * $p < 0.005$ compared to sham operated, non-immunized rats. † $p < 0.005$ compared to non-immunized rats with chronic biliary obstruction

operation or after 1 week's obstruction (Table VIII). When the uptake rate was corrected for the increase in liver volume the corrected colloidal uptake rate (cK_1) (formula (5)) was significantly decreased in 1

Table VIII. Uptake rate of $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid in the liver (K_1) and the extrahepatic RES (K_2) in rats with biliary obstruction and sham operation.

Groups	No. of rats	Colloid uptake rate (s^{-1}) ^a	
		Liver $\text{K}_1 \cdot 10^{-1}$	Extrahepatic RES $\text{K}_2 \cdot 10^{-2}$
A. Sham operation	8	0.16 $\pm$ 0.04	0.15 $\pm$ 0.03
B. 1 week obstruction	8	0.15 $\pm$ 0.04	0.15 $\pm$ 0.09
C. 3 week obstruction	8	0.14 $\pm$ 0.03	0.21 $\pm$ 0.06 ^b

^a Data are expressed as mean $\pm$ SD

^b $p < 0.005$ compared to both sham operation and 1 week biliary obstruction

week's biliary obstruction (4.3 ± 0.8) compared to sham operation (6.2 ± 0.3) ($p < 0.005$, t-test). The decrease of cK_1 was even more prominent in 3 weeks' obstruction (3.4 ± 0.3) compared to sham operation ($p < 0.005$) and 1 week's obstruction ($p < 0.025$, t-test) (Fig. 7). The total and relative

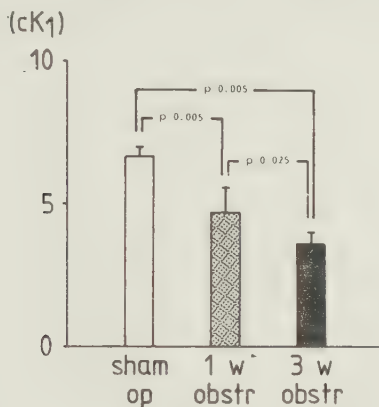


Fig. 7. Corrected colloidal uptake rate (cK_1) in rats with sham operation and biliary obstruction of 1 week and 3 weeks. Bars indicate mean $\pm$ SD.

ve activity distribution of $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid in per cent of injected activity are shown in Table IX. The relative activity in the liver was decreased in 1 week's obstruction compared to sham operation ($p < 0.005$,

Table IX. Activity distribution of $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid in per cent of injected activity in total organ and per gram tissue (mean $\pm$ SD) in rats with biliary obstruction and sham operation.

Groups	Liver		Spleen		Lung		Bone marrow
	%	%/gm	%	%/gm	%	%/gm	
A. Sham operation	89.5 $\pm$ 4.7	7.4 $\pm$ 0.6	3.7 $\pm$ 2.0	4.4 $\pm$ 2.4	0.39 $\pm$ 0.16	0.29 $\pm$ 0.12	0.17 $\pm$ 0.05
B. 1 week obstruction	89.4 $\pm$ 3.7	6.0 $\pm$ 1.0 ^a	3.4 $\pm$ 0.9	3.4 $\pm$ 0.8	0.54 $\pm$ 0.22	0.41 $\pm$ 0.16	0.18 $\pm$ 0.05
C. 3 week obstruction	83.3 $\pm$ 4 ^b	3.7 $\pm$ 0.7 ^b	4.7 $\pm$ 1.2 ^c	2.8 $\pm$ 0.7	0.98 $\pm$ 0.86	0.63 $\pm$ 0.52	0.30 $\pm$ 0.07 ^b

^a $p < 0.005$ compared to sham operation (group A)

^b $p < 0.005$ compared to both sham operation (group A) and 1 week biliary obstruction (group B)

^c $p < 0.025$ compared to 1 week biliary obstruction (group B)

t-test). The decrease of liver activity per gram tissue was even more pronounced in 3 weeks' biliary obstruction ($p < 0.005$, t-test). Total organ uptake rate of the liver in 3 weeks' obstruction was also significantly decreased compared to sham operation and 1 week's obstruction

($p < 0.005$, t-test). There were no significant differences in the colloidal uptake in the spleen and lungs per gram tissue between the three groups, although total organ uptake in the spleen was significantly increased in 3 weeks' obstruction compared to 1 week's obstruction ($p < 0.025$, t-test). A significant increase of bone marrow activity per gram tissue was noted in 3 weeks' obstruction compared to sham operation and 1 week's biliary obstruction ($p < 0.005$, t-test) (Table IX).

5. DISCUSSION

5.1. Methodological considerations

The spontaneous restoration of bile flow to the intestine following a single ligation of the common bile duct is a frequent finding in experimental animal models of obstructive jaundice (169). Animals are able to recanalize the bile duct even after a small resection of the common bile duct. Anatomical variations, such as low branching of hepatic ducts and high communication of pancreatic ducts with the CBD, preclude a substantial removal of the segment of the CBD. If the distance between the proximal cut end of the CBD and the distal cut end is short, it is quite likely that the proximal duct, when it becomes dilated, would fuse with the stump of the caudal part of the duct, leading to a recanalization of the CBD (78). Restoration of bile drainage is usually followed by the recovery of normal architecture of the liver in a few weeks (167). A sudden or gradual decrease of serum and urinary bilirubin reflects well the improvement of functional state of the liver (78). Since we are concerned with the RE function in complete biliary obstruction, appropriate animals were selected carefully, excluding any rats which had recanalized from the study. Thus, only those animals which revealed cystic dilatation of the proximal bile duct and with clinical jaundice were used in the experimental groups with biliary obstruction.

A retrograde intrabiliary (RI) injection technique was used in the present study to provide animal models of infection which originates in the biliary tree. Acquisition of bacteria in the bile in this way was assumed to closely resemble the natural way of ascending infection from the duodenum. This model gains an advantage over the conventional intravenous challenge system (161, 171) in that the capacity of the local immune system of the liver to prevent spreading of the bacteria to the blood stream can be tested. Furthermore, since this technique comprises the mode of transfer of bacteria across the epithelium of the biliary tree in the reabsorptive direction, it was regarded as suitable for the study of bacterial clearance from the bile (III).

In the experiments of bacterial challenge (I, II, V, VI), the CBD was punctured directly with a fine hypodermic needle to inject bacteria. This way of giving bacteria was preferred to the technique of catheterization to the CBD for injection of bacteria. Because, in the latter technique, it was anticipated that the injected bacteria might leak out at the time of removal of the catheter, and that if the catheter is retained in the body instead of being removed after injection of bacteria, it might act as a foreign body which may facilitate the growth of contaminated bacteria. The injection of bacteria was performed very slowly with a minimal pressure, however, due to the unpredictable pressure rise which may occasionally occur, a possibility of regurgitation of bacteria in the blood stream could not be excluded.

The volume of bacteria for RI injection was limited to 0.1 ml. In the previous studies on mechanism of cholestasis, volumes of solution far exceeding the maximum capacity of the biliary tree were injected (180-183). Although no electron microscopic evidence of damage to bile canali-

colitis reported to occur after RI injection of larger volumes of solution (182), there still existed the possibility of damage to the ductal system by the injection of large volumes. Furthermore, injection of some substances in volumes greater than 0.1 ml has been shown to lead to the complete absorption of the substance (184). Thus, absorption process might be variable according to the volume injected. Since we still do not know the absorption mechanism for bacteria, and since the distended capacity of the biliary tree has been shown to be around 120 μ l (185), it was regarded reasonable to inject 0.1 ml of bacterial solution intrabiliary in the present study.

RI injection of radiolabelled bacteria was performed via a catheter at a rate of 32.8 μ l/min (IV). This rate was chosen since preliminary experiments showed that a more rapid infusion increased the biliary pressure more than twice above the resting pressure in normal rats. Using the rate of 32.8 μ l/min, the biliary pressure promptly returned to the basal level after cessation of the infusion. If a substance is left in contact with the biliary tract epithelium for a longer period of time, it is suggested that absorption from the bile is increased (186). Thus, the relatively longer infusion time of 3.5 min to inject 118 μ l (IV) may have acted to enhance bacterial clearance from the biliary tree.

Radiolabelling of *E. coli* with ^{125}I in the present study is an application of the chloramine-T oxidation method of Hunter and Greenwood (187). This method has been used to label hormones (188), bacterial flagellins (189) and immunoglobulins (174). ^{125}I -labelling of bacteria, such as ^{125}I -labelled pneumococci (145) and ^{125}I -salmonella (190) have also been reported. Since the iodination is carried out in the presence of high concentrations of a powerful oxidizing agent, protein denaturation has been one of the drawbacks in this method (191). This problem, however, has been dealt with by the modified procedure employing far lower concentrations of chloramine-T and longer reaction times, maintaining efficient iodination and avoiding denaturation (174). Using this trace iodination method of proteins, efficiencies of iodination for most proteins have been shown to be between 40-90 % (174). In the present study, a mixture of 10^9 *E. coli* and radioactive iodide was allowed to react with 200 μ g of chloramine T for 5 min. The labelling efficiency was 20-30 %.

Due to the metabolic activity of the RES, the rapid clearance of ^{131}I -labelled albumin (156) or ^{131}I RE test lipid emulsion (192) has been shown to be followed by the reappearance of free iodide in the circulation during 10 to 15 min after injection. A release of free iodide may result from particle degradation or deiodination after RES uptake. Since deiodination of ^{125}I -labelled *E. coli* was therefore similarly anticipated, blood clearance study using ^{125}I -labelled *E. coli* was limited to 10 min following challenge in paper VI. Although there has not been any available data so far for comparison, the study of bacterial clearance from the bile in normal rats (III) showed a second peak of radioactivity occurring 20-30 min after injection of the bacteria. This might reflect released radiolabelled bacterial products, or metabolites from the breakdown of the microorganisms. Accordingly, bacterial recovery in bile was studied for 15 min in the paper (IV) to exclude the possibility of also counting bacterial metabolites. Data concerning radioactivity in the bile

later beyond limits of the range of bacteria in the bile therefore might indicate in the following days of a depression of bacterial metabolism or barrier of phagocytes. A significant decrease of radioactivity in the bile after 48 hours of periods of incubation (14), however, not only indicated the active excretion of bacteria in the bile during this period but also suggested that the fraction of excretion of bacterial metabolites was low.

Among several colloids used for determination of RE function (193), $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid has been well characterized in respect of size, shape, number, and labelling efficiency (175, 177). Since it is of utmost importance to use a highly standardized colloid for estimation of RE function (194, 195), the labelling efficiency was tested in each of the experiments with gel chromatography column scanning (177), and estimation of the particle size was performed by scanning electron microscopy (176) (VII). Thus, results of colloidal distribution in the present study cannot be attributed to variations in particle size and number of injected particles (196).

The conventional method of measuring plasma colloid clearance (127) is problematic in that only a few measuring points were used for calculation of the phagocytic index. This drawback has been overcome by the assumption of a two compartment model and a use of a scintillation camera technique (193), where calculation of uptake was made using the whole uptake curve, giving enough measuring points for appropriate calculations. Applying this technique in the present study (VII), separate measurements of hepatic and extrahepatic (mainly spleen) RE function could be done. The plasma colloid clearance technique conventionally used does not permit separate studies of the uptake by different RE organs.

5.2. RE function in biliary obstruction and biliary sepsis

In the present animal model of biliary sepsis, RI injection of bacteria is inevitably accompanied with simultaneous occlusion of the CBD, in order to avoid injected bacteria flowing down into the intestines, or leaking into the peritoneum, when standardization of the bacterial dose can not be achieved. Nevertheless, RI injection of *E. coli* did not cause any significant mortality in control rats. This forms a striking contrast to the increased susceptibility to the bacteria in rats with biliary obstruction of different durations (Table IV). Normal rats could obviously deal with the bacteria before any pathological changes due to biliary obstruction or bacterial infection occurred in the liver. On the other hand, an increased susceptibility to the RI-injected bacteria in rats with 3 days of biliary obstruction needs to be explained. Depression of RE function as early as 3 days after biliary obstruction has been considered unlikely (153-155, 159). Previous experiments in our laboratory using methyl palmitate and carrageenan to block RE function of the liver failed to increase the susceptibility to RI injection of bacteria in normal rats (Tanaka, unpublished data). Thus, the difference in the susceptibility to bacteria between the 3 days' obstructed rats and the control rats immediately after ligation of the CBD, points to the importance of the preexisting cholestasis, instead of RE function, in the mechanism of host defense failure.

Biliary obstruction of 3 days results in a several-fold increase in plasma level as well as liver contents of bile acids (197, 198). Bile acids are strong detergents and are known to damage hepatocytes (198). Thus, it is possible that bile acids play some role in the mechanism behind an increased susceptibility to bacteria in 3 days' jaundiced rats. Bile acids are shown to disturb human lymphocyte function (199-201). A similar mechanism of bile acid-mediated impairment of cellular immunity might occur in obstructed rats. Charcot-Gombault type of necrosis of hepatocytes, which is most prominent within 3 days of biliary obstruction (160), might facilitate regurgitation of bacteria to the blood stream leading to fulminant sepsis in these rats. Bile acids might also disturb killing mechanisms for bacteria (119). Whether any of these assumptions are truly involved need to be clarified.

On the other hand, a further increase of the susceptibility to bacteria in rats with prolonged biliary obstruction suggested a gradual deterioration of RE function according to the duration of obstructive jaundice. This is consistent with the progressive impairment of the liver function seen in animals with longstanding obstructive jaundice (202).

Although normal rats immediately after RI injection of bacteria cleared the bacteria, splenectomized (otherwise) normal rats could not resist RI injection of bacteria (V). This indicates a protective role of spleen following RI injection of bacteria. The challenge system in the present study concerns primarily the local defense mechanism, and secondarily systemic RE function to deal with the bacteria passing into the blood stream. Since almost all splenectomized rats after RI injection of bacteria survived more than 3 days after challenge, an initial protective mechanism against spilled bacteria, if any, seemed to work properly in the absence of the spleen. This may be illustrated by the fact that IV injection of the same dose of E. coli did not kill any of the splenectomized animals with or without biliary obstruction (Table V). However, overwhelming postsplenectomy sepsis by E. coli has been shown not to be rare (147) and splenectomy has been shown to lead to impairment of blood clearance of E. coli (149). The discrepancy in the results of IV injection of E. coli thus might be ascribed to the relatively low dose of the injected bacteria in the present study. The delayed course of sepsis in splenectomized rats therefore suggested that the bacteria had to grow and multiply in the bile during a few days to establish biliary sepsis after RI injection of bacteria. Using carbon colloids for the measurement of blood clearance rate, Lázár (154) and Holman and Rikkers (155) showed that the spleen did not influence granulopoietic activity in partially hepatectomized rats or biliary obstructed rats. However, results in the present study clearly demonstrated the importance of some splenic factor, which is vital for the protection against bacterial infection but not vital if the same numbers of bacteria are injected directly IV.

Bacterial clearance curve from the biliary tree (Fig. 3) was impressive in that the radioactivity in the bile decreased exponentially in the first 15 minutes in control rats, whereas the clearance was greatly impaired in rats with chronic biliary obstruction (III). A decrease in percentage recovery of radioactivity in the bile after 1 hour's incubation of bacteria in the bile in control rats was concomitant with an increase of retention

of bacteria in the liver (IV). This decrease in recovery produced by prolongation of the occlusion time of biliary tree is in accordance with the finding of Olson and Fujimoto (186). However, a decrease in recovery of bacteria in the bile at 1 hour incubation in obstructive jaundice was not accompanied by a proportional increase of retention of bacteria in the liver (IV). Morphological changes in cholestasis, such as rupture of bile canaliculi (203), increased permeability of tight junctions (73, 74), increased reversed pinocytosis by hepatocytes (180), or distended intercellular space in the bile ducts and ductules (182, 204), may facilitate a regurgitation of injected bacteria out of the biliary tree. However, the results of bacterial clearance from the bile (III, IV) showed a decreased early clearance rate and a retention of more than 70 % of injected bacteria in the extrahepatic bile tree. This clearly indicates that the mechanism of septicemia of biliary origin is not determined by a simple sequence of an increased intrabiliary pressure followed by a regurgitation of a large proportion of the bacteria in the systemic circulation. It is probable therefore that the impaired capacity of the obstructed liver to take up bacteria from the bile is a crucial factor in the development of biliary septicemia in obstructive jaundice.

The bacterial clearance rate from the blood (K) was not different between sham operated rats and chronic biliary obstructed rats (VI). Uptake rate of $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid in the liver (K_1) was also not different between sham operated rats, 1 week obstructed rats and 3 weeks' obstructed rats (VII). Particle ingestion by RE cells has been suggested to be influenced by blood flow (205) and opsonins (122). Relative increase of liver weight in biliary obstruction (VII) suggests an increase of blood flow to the liver in obstructed animals. Aronsen et al. (206) found that this is so until the 3rd week of biliary obstruction in dog. Since comparison of particle clearance rate between different animals with different liver weight is useless unless data are corrected for the difference of blood flow, the corrected colloidal uptake rate (cK_1) was calculated in the paper (VII). Corrected colloidal uptake rate (cK_1) corresponds to the corrected phagocytic index (α) originally described by Biozzi (127), who placed a particular importance on the relative blood flow in the liver and spleen. Thus, RE function measured by cK_1 was obviously depressed in 1 week's as well as 3 weeks' biliary obstruction (VII). The results, however, are in contrast to previous studies of Halpern et al. (153), who showed an increased activity of RES until 5 weeks of biliary obstruction. Holman and Rikkers (155) also showed a diverse result in which RE depression was demonstrated only after 3 weeks' biliary obstruction. These contradictory results might be ascribed to the differences in physico-chemical properties of the test substance used (207), or to the experimental procedures for producing biliary obstruction. A significant increase of relative liver weight in 1 week obstruction is a reflection of the completeness of the biliary obstruction. This finding was obscure in the previous studies. Another explanation can be the differences in the technique for measurement of colloidal uptake rate. Suppression of colloid uptake in the liver is often accompanied by an increased colloidal uptake in the extrahepatic RES. However, a classical technique of plasma colloid clearance does not permit separate studies of the uptake by different RE organs. The corrected phagocytic index using plasma clearance

rate of colloid in the earlier studies therefore might have masked a depression of RE function in the liver. Owing to the technique applied in the present study (VII), a subtle change in RE function of the liver may be detected as early as 1 week after biliary obstruction.

The RES phagocytoses preferentially electronegative colloids and particles. Bacteria do not appear to be extracted quite as efficiently as colloids (208). Thus, the percentage distribution of uptake rate of test substance by different organs was different in study (VI) from that in study (VII). Furthermore, different particle size (i.e., bacteria and $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid) is considered to contribute to the different pattern of organ distribution of particles. Using ^{125}I -labelled E. coli (VI), a decreased liver and spleen uptake and an increased lung uptake per unit weight was demonstrated in obstructive jaundice. Total organ localization of bacteria in lung was also increased. The intravenous injection of a large number of bacteria is known to cause many polymorphs to adhere to the endothelium of small blood vessels in the lung, where they ingest bacteria (209). During severe RE depression, it is suggested that bacteremia can cause microvascular embolization, endothelial injury, altered capillary permeability, endothelial swelling, interstitial oedema and disrupted gas exchange (138). The rats with chronic biliary obstruction in the present study therefore might become susceptible to the bacteria through a potentially lethal positive feed-back mechanism towards organ failure. On the other hand, activity distribution of $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid (VII) clearly show a decreased uptake in the liver after 3 weeks' biliary obstruction. The total organ colloid uptake reflects the functioning mass of macrophages in the corresponding organ. Thus, the decreased colloidal uptake in the liver as a total organ strongly suggested a depression of the hepatic macrophage function after 3 weeks' biliary obstruction. Conversely, the colloidal uptake rate of the extrahepatic RES (K_2) was shown to be increased after 3 weeks' obstruction (VII). However, the increased activity of the extrahepatic RES is inadequate to compensate the depressed hepatic RE function. The total RES function of the animal therefore is considered diminished.

The increased localization of bacteria to the lung in chronic biliary obstruction in study (VI) was in accordance with a previous report (159). However, the finding that active immunization in rats with chronic biliary obstruction significantly promoted lung uptake of bacteria needs consideration. Lanser and Saba (210) reported that the localization of bacteria in the lung following colloid-induced RE blockade was further increased by administration of fibronectin. They suggested that fibronectin increased the number of neutrophils margined in the lung following blockade and thereby provided an increased bacterial uptake in the lung. A similar mechanism of neutrophil-mediated lung localization of bacteria might explain the vaccine-effect. Thus, the advantage of the improved opsonization provided by active immunization was considered to be neutralized by the less beneficial effect of overloading of the lungs with bacteria. Results indicated that further studies are required to prove the beneficial effect of immunotherapy in chronic biliary obstruction.

5.3. Clinical considerations and future aspects

Diagnostic cholangiography always carries the hazard of causing cholangio-venous reflux. In order to avoid delivering infected bile to the systemic circulation as much as possible, cholangiography injections should be performed with minimal pressure. Insertion of a catheter for PTBD is possible under ultrasonic guidance without initial thin needle cholangiography (211). Taking advantage of the ultrasonically guided PTBD technique, drainage of stagnant bile for several days without the need to delineate the biliary tree with contrast media may be beneficial. Cholangiography can be done after an ample decompression of the bile duct is achieved with more safety than with the conventional technique of PTBD. Using a fine catheter for PTBD, thick pus may be difficult to drain. Using a larger-bore catheter, however, complications will not be negligible (94-96). Ultrasonography helps avoiding inadvertent puncture of the intrahepatic portal vein with the thick needle (211), thus minimizing the risk of producing a large cholangio-venous communication.

As any preoperative manipulation to the bile duct has the potential for causing bacteria in the CBD to spill over into the systemic circulation, a policy of initial operative treatment without preoperative decompression technique should be re-evaluated, unless the case is urgent with septic deterioration.

Measurements of RE function available now, carry the risk of blocking the RES with the test colloids. In order to overcome this problem, further refinement of the test substance is required. Or some new in vitro tests to measure RE function should be developed.

An increased susceptibility to septicemia in the early period of biliary obstruction suggests the benefit of early decompression of the bile duct. However, decompression procedures hold the potential risk of infection because of the catheter or stent. Bilirubin itself even in high concentration seems not to give a deleterious effect to any extent. So, the timing and opportunity for decompression should be balanced with the severity of the disease and the benefit of the decompression.

Systemic antibiotic therapy is still not effective. Topical administration of drugs at the time of decompression procedures may be useful, especially if suitable access to the biliary system is available after the decompression.

Whether stimulation of RES can be achieved in obstructive jaundice requires further study. In order to support or protect from a further decrease in RE function during the course of obstructive jaundice, immunotherapy might be of help. However, at the moment, the side effects seem to overwhelm the benefits. Thus, further improvements in the techniques of active and passive immunization and methods of administration are necessary.

6. SUMMARY AND CONCLUSIONS

- (1) Both the presence of extrahepatic biliary obstruction and the introduction of bacteria into the biliary system were shown to be prerequisites for production of biliary sepsis.
- (2) RI injection of E. coli demonstrated an increased susceptibility to bacteria starting as early as 3 days after biliary obstruction in rats. Animals with longstanding jaundice were more susceptible than those with a shorter duration of biliary obstruction.
- (3) The spleen was shown to play a crucial role for protection against fatal outcome in biliary sepsis.
- (4) Bacterial clearance capacity of the liver from the biliary tree was greatly impaired in rats with chronic biliary obstruction.
- (5) RE function evaluated by measuring the biokinetics of $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid demonstrated a depression of RE activity of the liver in biliary obstruction.
- (6) The circulatory specific antibodies in rats with chronic biliary obstruction increased the uptake of bacteria in the lungs.

7. ACKNOWLEDGEMENTS

I wish to express my sincere thanks to all co-workers and to all those who in any way contributed to and influenced the completion of the present thesis.

I also express my sincere gratitude to:

Mrs Monica Radnell and Inger Mårtensson for their technical assistance.

Miss Mona Magnusson and Ingrid Christensson for bacterial preparation.

Dr Malcolm Puntis for revision of the English language.

Miss Eva Edestad for typing and illustration.

Mr Pehr Johnson for printing.

8. REFERENCES

1. Thompson, J.E., Thompson, R.K., Longmire, W.P.: Factors in management of acute cholangitis. *Ann. Surg.* 195:137-145, 1982.
2. Wolbach, S.B., Saiki, T.: A new anaerobic spore-bearing bacterium commonly present in the livers of healthy dogs, and believed to be responsible for many changes attributed to aseptic autolysis of liver tissue. *J. med. Res.* 21:267-278, 1909.
3. Schweinburg, F.B., Sylvester, E.M.: Bacteriology of the healthy experimental animal. *Proc. Soc. Exp. Biol. Med.* 82:527-530, 1953.
4. Lykkegaard Nielsen, M., Justesen, T., Asnaes, S., Stage, J.G., Nielsen, O.V.: Anaerobic and aerobic bacteriological study of the liver and biliary tract in healthy laboratory animals: Investigations in rabbits, guinea pigs, mice, and pigs. *Danish Med. Bull.* 22:159-164, 1975.
5. Sborov, V.M., Morse, W.C., Giges, B., Jahnke, E.J.: Bacteriology of the human liver. *J. Clin. Invest.* 31:986-992, 1952.
6. Scott, A.J.: Progress report. Bacteria and disease of the biliary tract. *Gut* 12:487-492, 1971.
7. Edlund, Y.A., Mollstedt, B.O., Ouchterlony, Ö.: Bacteriological investigation of the biliary system and liver in biliary tract disease correlated to clinical data and microstructure of the gallbladder and liver. *Acta chir. scand.* 116:461-476, 1958/59.
8. Lykkegaard Nielsen, M., Justesen, T., Asnaes, S.: Anaerobic bacteriological study of the human liver - with a critical review of the literature. *Scand. J. Gastroent.* 9:671-677, 1974.
9. Shimada, K., Inamatsu, T., Yamashiro, M.: Anaerobic bacteria in biliary disease in elderly patients. *J. Infect. Dis.* 135:850-854, 1977.
10. Hatfield, A.R.W., Leung, T., Ahmet, Z., Williams, J.D.: The microbiology of direct bile sampling at the time of endoscopic retrograde cholangiopancreatography. *J. Infect.* 4:119-125, 1982.
11. Maddocks, A.C., Hilson, G.R.F., Taylor, R.: The bacteriology of the obstructed biliary tree. *Ann. Roy. Coll. Surg.* 52:316-319, 1973.
12. Keighley, M.R.B., Lister, D.M., Jacobs, S.I., Giles, G.R.: Hazards of surgical treatment due to microorganisms in the bile. *Surgery* 75: 578-583, 1974.
13. Bourgault, A., England, D.M., Rosenblatt, J.E., Forgacs, P., Bieger, C.: Clinical characteristics of anaerobic bactibilia. *Arch. Intern. Med.* 139:1346-1349, 1979.

14. Cox, J.L., Helfrich, L.R., Pass, H.I., Osterhaut, S., Shingleton, W.W.: The relationship between biliary tract infections and post-operative complications. *Surg. Gynecol. Obstet.* 146:233-236, 1978.
15. Pitt, H.A., Postier, R.G., Cameron, J.L.: Biliary bacteria. Significance and alterations after antibiotic therapy. *Arch. Surg.* 117:445-449, 1982
16. Roberts, A.B., Finlay-Jones, J.J., Watts, J.M.: Infection in biliary surgery: Anaerobic organisms and acute cholecystitis. *Surg. Gastroenterol.* 1:183-189, 1982.
17. Flemma, R.J., Flint, L.M., Osterhout, S., Shingleton, W.W.: Bacteriologic studies of biliary tract infection. *Ann. Surg.* 166:563-572, 1967.
18. Scott, A.J., Khan, G.A.: Origin of bacteria in bile duct bile. *Lancet* ii:790-792, 1967.
19. Jackaman, F.R., Hilson, G.R.F., Lord Smith of Marlow: Bile bacteria in patients with benign bile duct stricture. *Br. J. Surg.* 67:329-332, 1980.
20. Claesson, B., Holmlund, D., Mätzsch, T.: Biliary microflora in acute cholecystitis and the clinical implications. *Acta chir. scand.* 150: 229-237, 1984.
21. Dye, M., Macdonald, A., Smith, G.: The bacterial flora of the biliary tract and liver in man. *Br. J. Surg.* 65:285-287, 1978.
22. Farnell, M.B., van Heerden, J.A., Beart, R.W.: Elective cholecystectomy. The role of biliary bacteriology and administration of antibiotics. *Arch. Surg.* 116:537-540, 1981.
23. Lykkegaard Nielsen, M., Moesgaard, F., Justesen, T., Scheibel, J.H., Lindenberg, S.: Wound sepsis after elective cholecystectomy. Restriction of prophylactic antibiotics to risk groups. *Scand. J. Gastroent.* 16:937-940, 1981.
24. Lykkegaard Nielsen, M., Justesen, T.: Anaerobic and aerobic bacteriological studies in biliary tract disease. *Scand. J. Gastroent.* 11: 437-446, 1976.
25. O'Connor, M.J., Schwartz, M.L., McQuarrie, D.G., Sumner, H.W.: Cholangitis due to malignant obstruction of biliary outflow. *Ann. Surg.* 193:341-345, 1981.
26. Wise, L., Pizzimbono, C., Dehner, L.P.: Periampullary cancer: a clinicopathologic study of sixty-two patients. *Am. J. Surg.* 131:141-148, 1976.
27. Keighley, M.R.B.: Microorganisms in the bile. A preventable cause of sepsis after biliary surgery. *Ann. Roy. Coll. Surg.* 59:328-334, 1977.

28. McPherson, G.A.D., Blenkarn, J.I., Nathanson, B., Bowley, N.B., Benjamin, I.S., Blumgart, L.H.: The significance of bacteria in external biliary drainage systems: a possible role for antisepsis. *J. Clin. Surg.* 1:22-26, 1982.
29. Keighley, M.R.B., Drysdale, R.B., Quoraishi, A.H., Burdon, D.W., Alexander-Williams, J.: Antibiotic treatment of biliary sepsis. *Surg. Clin. North Amer.* 55:1379-1390, 1975.
30. Mason, G.R.: Bacteriology and antibiotic selection in biliary tract surgery. *Arch. Surg.* 97:533-537, 1968.
31. Chetlin, S.H., Elliott, D.W.: Biliary bacteremia. *Arch. Surg.* 102: 303-307, 1971.
32. Engström, J., Groth, C.G., Lundh, G., Lönnqvist, B.: Infectious complications after surgery for biliary calculus: The significance of bacteria in the biliary tract. *Acta chir. scand.* 138:357-361, 1972.
33. Gordon-Taylor, G., Whitby, L.E.H.: The incidence of anaerobic infections in the gallbladder. *Br. J. Surg.* 19:619-621, 1932.
34. Schottenfeld, L.E.: Anaerobic infection of the biliary tract. A review and analysis of thirteen cases from the literature with the addition of eight new cases. *Surgery* 27:710-719, 1950.
35. Pyrtek, L.J., Bartus, S.H.: *Clostridium welchii* infection complicating biliary tract surgery. *New Engl. J. Med.* 266:689-693, 1962.
36. McLeish, A.R., Keighley, R.B., Bishop, H.M., Burdon, D.W., Path, M.R.C., Quoraishi, A.H., Dorricott, N.J., Oates, G.D., Alexander-Williams, J.: Selecting patients requiring antibiotics in biliary surgery by immediate gram stains of bile at operation. *Surgery* 81: 473-478, 1977.
37. Keighley, M.R.B., Flinn, R., Alexander-Williams, J.: Multivariate analysis of clinical and operative findings associated with biliary sepsis. *Br. J. Surg.* 63:528-531, 1976.
38. Patek, P.R., Mignard, V.A., Bernick, S.: Age changes in structure and responses of reticuloendothelial cells of rat liver. *J. Reticuloendothelial Soc.* 4:211-218, 1967.
39. Seth, V., Chandra, R.K.: Opsonic activity, phagocytosis, and bactericidal capacity of polymorphos in undernutrition. *Arch. Dis. Child.* 47:282-284, 1972.
40. Cameron, R., Hou, P.C.: In *Biliary Cirrhosis* (edited by Cameron, R. and Wright, G.P.), p. 82, Edinburgh, 1962.
41. Prytz, H., Holst-Christensen, J., Korner, B., Liehr, H.: Portal venous and systemic endotoxaemia in patients without liver disease and systemic endotoxaemia in patients with cirrhosis. *Scand. J.*

42. Jacob, A.I., Goldberg, P.K., Bloom, N., Degenshein, G.A., Kozinn, P.J.: Endotoxin and bacteria in portal blood. *Gastroenterology* 92: 1268-1270, 1977.
43. Orloff, M.J., Peskin, G.W., Ellis, H.L.: A bacteriologic study of human portal blood: Implications regarding hepatic ischemia in man. *Ann. Surg.* 148:738-746, 1958.
44. Wolochow, H., Hildebrand, G.J., Lamanna, C.: Translocation of microorganisms across the intestinal wall of the rat: Effect of microbial size and concentration. *J. Infect. Dis.* 166:523-528, 1966.
45. Bennion, R.S., Wilson, S.E., Russell, R.A.: Early portal anaerobic bacteremia in mesenteric ischemia. *Arch. Surg.* 119:181-155, 1984.
46. Freedman, L.R., Goldenberg, I.S.: The relationship between bacterial infection of the hepato-biliary system and common bile duct obstruction in man. *Yale J. Biol. Med.* 35:318-327, 1963.
47. Cardoso, V., Pimenta, A., de Fonseca, C., Rodrigues, J.S., Vaz, M.J.M.: The effect of cholestasis on hepatic clearance of bacteria. *World J. Surg.* 6:330-334, 1982.
48. Dineen, P.: The importance of the route of infections in experimental biliary tract obstruction. *Surg. Gynecol. Obstet.* 119:1001-1008, 1964.
49. Gorbach, S.L., Plaut, A.G., Nahas, L., Weinstein, L.: Studies of intestinal microflora. II. Microorganisms of the small intestine and their relations to oral and fecal flora. *Gastroenterology* 53:856-867, 1967.
50. Smith, H.W.: Observations on the flora of the alimentary tract of animals and factors affecting its composition. *J. Path. Bact.* 89:95-122, 1965.
51. Kalser, M.H., Cohen, R., Arteaga, I., Yawn, E., Mayoral, L., Hoffert, W.R., Frazier, D.: Normal viral and bacterial flora of the human small and large intestine. *New Engl. J. Med.* 274:500-505, 1966.
52. Engström, J., Hellström, K., Högman, L., Lönnqvist, B.: Microorganisms of the liver, biliary tract and duodenal aspirates in biliary diseases. *Scand. J. Gastroent.* 6:177-182, 1971.
53. Prasar, B.S., Shiner, M., McLeod, G.M.: Studies on the intestinal flora. I. The bacterial flora of the gastrointestinal tract in healthy and achlorhydric persons. *Gastroenterology* 56:71-79, 1969.
54. Dixon J.M.: The fate of bacteria in the small intestine. *J. Path. Bact.* 79:131-140, 1960.

55. Griffen, W.O., Richardson, I.D., Medley, E.: Prevention of small bowel contamination by ileocaecal valve. *Sth. Afr. Med. J.* 64:1056-1058, 1971.
56. Goldsworthy, N.E., Florey, H.: Some properties of mucus with special reference to its antibacterial functions. *Br. J. exp. path.* 11:192-208, 1930.
57. Meynell, G.G.: Antibacterial mechanisms of the mouse gut. II. The role of Eh and volatile fatty acids in the normal gut. *Br. J. exp. path.* 44:209-219, 1963.
58. Brown, W.R., Newcombe, R.W., Ishizaka, K.: Proteolytic degradation of exocrine and serum immunoglobulins. *J. Clin. Invest.* 49:1374-1380, 1970.
59. Floch, M.M., Gershengoren, W., Elliott, S., Spiro, M.M.: Bile acid inhibition of the intestinal microflora - A function of simple bile acids? *Gastroenterology* 61:228-233, 1971.
60. Menguy, R., Koger, E.: Mechanism of inhibition of gastric secretion in the rat following bile duct ligation. *Proc. Soc. Exp. Biol. Med.* 101:666-668, 1959.
61. Silen, W., Skillman, J.J., Hein, M., Harper, H.A.: The effect of biliary obstruction upon canine gastric secretory activity. *J. Surg. Res.* 11:197-200, 1962.
62. Arnot, R.S., Terblanche, J., Hickman, R.H., Barbezat, G.O., Louw, J.H.: Acid secretion in the bile duct-ligated pig. *Sth. Afr. med. J.* 56:606-608, 1979.
63. Skalkas, G., Simopoulos, C., Karatzas, G., Alexious, D., Karidakis, P., Kostakis, A., Sechas, M.: Baisse de l'acidité gastrique après ligature du cholédoque chez le lapin. *J. Chir. (Paris)* 117:723-725, 1980.
64. Huang, T., Bass, J.A., Williams, R.D.: The significance of biliary pressure in cholangitis. *Arch. Surg.* 98:629-632, 1969.
65. Kinoshita, H., Hirohashi, K., Igawa, S., Nagata, E., Sakai, K.: Cholangitis. *World J. Surg.* 8:963-969, 1984.
66. Mallet-Guy, P.: Value of preoperative manometric and roentgenologic examination in the diagnosis of pathologic changes and functional disturbances of the biliary tract. *Surg. Gynecol. Obstet.* 94:385-393, 1952.
67. Mixer, H.W., Rigler, L.G., Oddone, M.V.G.: Experimental studies on biliary regurgitation during cholangiography. *Gastroenterology* 9: 64-80, 1947.

68. Hultborn, A., Jacobsson, B., Rosengren, B.: Cholangiovenous reflux during cholangiography. An experimental and clinical study. *Acta chir. scand.* 123:111-124, 1962.
69. Charcot, J.M.: *Leçons sur les maladies du foie des voies filiales et des reins*. Paris: Faculté de Médecine de Paris, 1877.
70. Jacobsson, B., Kjellander, J., Rosengren, B.: Cholangiovenous reflux. An experimental study. *Acta chir. scand.* 123:316-321, 1962.
71. Robenek, H., Herwig, J., Themann, H.: The morphologic characteristics of intercellular junctions between normal human liver cells and cells from patients with extrahepatic cholestasis. *Am. J. Pathol.* 100:93-114, 1980.
72. Schatzki, P.F.: Bile canaliculus and space of Disse. Electron microscopic relationships as delineated by Lanthanum. *Lab. Invest.* 20: 87-93, 1969.
73. Metz, J., Aoki, A., Merlo, M., Forssmann, W.G.: Morphological alterations and functional changes of interhepatocellular junctions induced by bile duct ligation. *Cell Tiss. Res.* 182:299-310, 1977.
74. De Vos, R., Desmet, V.J.: Morphologic changes of the junctional complex of the hepatocytes in rat liver after bile duct ligation. *Br. J. exp. path.* 59:220-227, 1978.
75. Hampton, J.C.: Electron microscopic study of extrahepatic biliary obstruction in the mouse. *Lab. Invest.* 10:502-513, 1961.
76. Koga, A., Todo, S.: Morphological and functional changes in the tight junctions of the bile canaliculi induced by bile duct ligation. *Cell Tiss. Res.* 195:267-276, 1978.
77. Bockman, D.E.: Route of flow and micropathology resulting from retrograde intrabiliary injection of india ink and ferritin in experimental animals. *Gastroenterology* 67:324-332, 1974.
78. Trams, E.G., Symeonidis, A.: Morphologic and functional changes in the livers of rats after ligation or excision of the common bile duct. *Am. J. Pathol.* 33:13-27, 1957.
79. Reynolds, B.M., Dargan, E.L.: Acute obstructive cholangitis. A distinct clinical syndrome. *Ann. Surg.* 159:299-303, 1959.
80. Rogers, L.: Biliary abscesses of liver with operation. *Br. Med. J.* 2:706, 1903.
81. Hauptert, A.P., Carey, L.C., Evans, W.E., Ellison, E.H.: Acute suppurative cholangitis. Experience with 15 consecutive cases. *Arch. Surg.* 94:460-468, 1966.

82. Glenn, F., Moody, F.G.: Acute obstructive suppurative cholangitis. Surg. Gynecol. Obstet. 113:265-273, 1961.
83. Ostermiller, W., Thompson, P.J., Carter, R., Hinshaw, D.B.: Acute obstructive cholangitis. Arch. Surg. 90:392-395, 1965.
84. Dow, R.W., Lindenauer, S.M.: Acute obstructive suppurative cholangitis. Ann. Surg. 169:272-276, 1969.
85. Boey, J.H., Way, L.W.: Acute cholangitis. Ann. Surg. 191:264-270, 1980.
86. Glenn, F., Evans, J.A., Mujahed, Z.: Percutaneous transhepatic cholangiography. Ann. Surg. 156:450-462, 1962.
87. Weichel, K.L.: Percutaneous transhepatic cholangiography. Techniques and application. Acta chir. scand. (suppl.) 330:1-99, 1964.
88. Kaude, J.V., Weidemier, C.H., Agee, O.F.: Decompression of bile ducts with the percutaneous transhepatic techniques. Radiology 93:69-71, 1969.
89. Molnar, W., Stockum, A.E.: Relief of obstructive jaundice through percutaneous transhepatic catheter - A new therapeutic method. Am. J. Radiol 122:356-367, 1974.
90. Takada, T., Hanyu, F., Kobayashi, S., Uchida, Y.: Percutaneous transhepatic cholangial drainage: Direct approach under fluoroscopic control. J. Surg. Oncol. 8:83-97, 1976.
91. Nakayama, T., Ikeda, A., Okuda, K.: Percutaneous transhepatic drainage of the biliary tract. Technique and results in 104 cases. Gastroenterology 74:554-559, 1978.
92. Ferrucci, J.T., Mueller, P.R., Harbin, W.P.: Percutaneous transhepatic biliary drainage. Radiology 135:1-13, 1980.
93. Mori, K., Misumi, A., Sugiyama, M., Okabe, M., Matsuoaka, T., Ishii, J., Akagi, M.: Percutaneous transhepatic bile drainage. Ann. Surg. 185:111-115, 1977.
94. McPherson, G.A.D., Benjamin, I.S., Habib, N.A., Bowley, N.B., Blumgart, L.H.: Percutaneous transhepatic drainage in obstructive jaundice: advantages and problems. Br. J. Surg. 69:261-264, 1982.
95. Hatfield, A.R.W., Tobias, R., Terblanche, J., Gierwood, A.H., Fataar, S., Harries-Jones, R., Kernoff, L., Marks, I.N.: Preoperative external biliary drainage in obstructive jaundice. A prospective controlled clinical trial. Lancet ii:896-899, 1982.
96. Nilsson, U., Evander, A., Ihse, I., Lunderquist, A., Mocibob, A.: Percutaneous transhepatic cholangiography and drainage. Risks and complications. Acta Radiol. 24:433-439, 1983.

97. Blenkarn, J.I., McPherson, G.A.D., Blumgart, L.H.: An improved system for external biliary drainage. *Lancet* ii:781-782, 1981.
98. Blenkarn, J.I., McPherson, G.A.D., Blumgart, L.H.: Septic complications of percutaneous transhepatic biliary drainage. *Am. J. Surg.* 147:318-321, 1984.
99. Norlander, A., Kalin, B., Sundblad, R.: Effect of percutaneous transhepatic drainage upon liver function and postoperative mortality. *Surg. Gynecol. Obstet.* 155:161-166, 1982.
100. McPherson, G.A.D., Benjamin, I.S., Hodgson, H.J.F., Bowley, N.B., Allison, D.J., Blumgart, L.H.: Preoperative percutaneous transhepatic biliary drainage: the results of a controlled trial. *Br. J. Surg.* 71:371-375, 1984.
101. Wardle, E.N., Wright, N.A.: Endotoxin and acute renal failure associated with obstructive jaundice. *Br. Med. J.* 4:472-474, 1970.
102. Bailey, M.E.: Endotoxin, bile salts and renal function in obstructive jaundice. *Br. J. Surg.* 63:774-778, 1976.
103. Ingoldby, C.J., McPherson, G.A.D., Blumgart, L.H.: Endotoxemia in human obstructive jaundice. Effect of polymyxin B. *Am. J. Surg.* 147:766-771, 1984.
104. Burcharth, F., Jensen, L.I., Olesen, K.: Endoprosthesis for internal drainage of the biliary tract. *Gastroenterology* 77:133-137, 1979.
105. Hoevels, J., Ihse, I.: Percutaneous transhepatic insertion of a permanent endoprosthesis in obstructive lesions of the extrahepatic bile ducts. *Gastrointest. Radiol.* 4:367-377, 1979.
106. Huibregtse, K., Haverkamp, H.J., Tytgat, G.N.: Transpapillary positioning of a large 3.2 mm biliary endoprosthesis. *Endoscopy* 13:217-219, 1981.
107. Mortimer, P.R., Mackie, D.B., Haynes, S.: Ampicillin levels in human bile in the presence of biliary tract disease. *Br. Med. J.* 3:88-89, 1969.
108. Khan, G.A., Scott, A.J.: The place of rifamycin-B-Diethylamide in the treatment of cholangitis complicating biliary obstruction. *Br. J. Pharmac. Chemother.* 31:506-512, 1967.
109. Burke, J.F.: The effective period of preventive antibiotic action in experimental incisions and dermal lesions. *Surgery* 50:161-168, 1961.
110. Stone, H.H., Hooper, C.A., Kolb, L.D., Geheber, C.E., Dawkins, E.J.: Antibiotic prophylaxis in gastric, biliary and colonic surgery. *Ann. Surg.* 184:443-452, 1976.

111. Garrod, L.P.: Causes of failure in antibiotic treatment. *Br. Med. J.* 4:473-476, 1972.
112. Kaufman, Z., Engelberg, M., Eliashiv, A., Reiss, R.: Systemic prophylactic antibiotics in elective biliary surgery. *Arch. Surg.* 119: 1002-1004, 1984.
113. Keighley, M.R.B., McLeish, A.R., Bishop, H.M., Burdon, D.W., Path, M.R.C., Quoraishi, A.H., Oates, G.D., Dorricott, N.J., Alexander-Williams, J.: Identification of the presence and type of biliary microflora by immediate gram strains. *Surgery* 81:469-472, 1977.
114. Beinfeld, M.S., Hayes, R.L.: Use of intraoperative gram stain during cholecystectomy. *Am. J. Surg.* 137:773-774, 1979.
115. Dooley, J.S., Hamilton-Miller, J.M.T., Brumfitt, W., Sherlock, S.: Antibiotics in the treatment of biliary infection. *Gut* 25:988-998, 1984.
116. Metchnikoff, I.: Lectures on the comparative pathology of inflammation. Kegan Paul, Trench, Trübner & Co., London (1893). Reprinted, Dover Publ. Inc., New York, pp. 137-143, 1968.
117. Alexander, J.W.: Host defense mechanisms against infection. *Surg. Clin. North Am.* 52:1367-1378, 1972.
118. Root, R.K., Cohen, M.S.: The microbicidal mechanisms of human neutrophils and eosinophils. *Rev. Inf. Dis.* 3:565-598, 1981.
119. Babior, B.M.: Oxygen-dependent microbial killing by phagocytes. *New Engl. J. Med.* 298:659-668, 1978.
120. Thorne, K.J.I., Oliver, R.C., Barrett, A.J.: Lysis and killing of bacteria by lysosomal proteinases. *Infect. Immun.* 14:555-635, 1976.
121. Aschoff, L.: I. Morphologie des reticulo-endothelialen Systems. In: Kraus, F., Meyer, E., Minkowski, O., Müller, F.R., Sahli, H., Schittenhelm, A., Czerny, A., Heubner, O., Langstein, L. (eds.) *Ergebnisse der Inneren Medizin und der Kinderheilkunde*. Julius Springer, Berlin, 1924.
122. Saba, T.M.: Physiology and Physiopathology of the reticuloendothelial system. *Arch. Int. Med.* 126:1031-1052, 1970.
123. van Furth, R., Cohn, Z.A., Hirsch, J.G., Spector, W.G., Langevoort, H.L.: The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. *Bull. W.H.O.* 46: 845-852, 1972.
124. van Furth, R., Langevoort, H.L., Schaberg, A.: Mononuclear phagocytes in human pathology: proposal for an approach to improved classification. In *Mononuclear Phagocytes in Immunity, Infection, and Pathology*. R. van Furth, editor. Blackwell Scientific Publications

Ltd, Oxford, England

125. Crofton, R.W., Martina, M.C., Diesselhoff, D., van Furth, R.: The origin, kinetics, and characteristics of the Kupffer cells in the normal steady state. *J. Exp. Med.* 148:1-17, 1978.
126. Tanner, A.R., Arthur, M.J.P., Wright, R.: Macrophage activation, chronic inflammation and gastrointestinal disease. *Gut* 25:760-783, 1984.
127. Biozzi, G., Benacerraf, B., Halpern, B.N.: Quantitative study of the granulopoietic activity of the reticuloendothelial system. II; A study of the kinetics of the granulopoietic activity of the RES in relation to the dose of carbon injected. Relationship between the weight of the organ and their activity. *Br. J. exp. path.* 34:441-457, 1953.
128. Benacerraf, B., Sebestyen, M.M., Schlossman, S.: A quantitative study of the kinetics of blood clearance of ³²P-labeled Escherichia coli and Staphylococci by the reticuloendothelial system. *J. Exp. Med.* 110:27-48, 1959.
129. Kerby, G.P., Holland, B.C., Martin, S.P.: The quantitative estimation of the removal of bacteria from the blood by the various organs of the immunized animals. *J. Immunol.* 64:123-129, 1950.
130. Rogers, D.E.: Host mechanisms which act to remove bacteria from the blood stream. *Bact. Rev.* 24:50-66, 1960.
131. Wardlaw, A.C., Howard, J.G.: A comparative survey of the phagocytosis of different species of bacteria by Kupffer cells. *Br. J. exp. path.* 40:113-117, 1959.
132. Wright, A.E., Douglas, B.R.: An experimental investigation of the role of the blood fluids in connection with phagocytosis. *Proc. Roy. Soc.* 72:357-370, 1903.
133. Rowley, D.: Phagocytosis. *Adv. Immun.* 2:241-264, 1962.
134. Jenkin, C.R., Rowley, D.: The role of opsonins in the clearance of living and inert particles by the cells of the reticuloendothelial system. *J. Exp. Med.* 114:363-374, 1961.
135. Saba, T.M., Di Luzio, N.R.: Reticuloendothelial blockade and recovery as a function of opsonic activity. *Am. J. Physiol.* 216:197-205, 1969.
136. Alexander, J.W., McClellan, M.A., Ogle, C.K., Ogle, J.D.: Consumptive opsoninopathy: Possible pathogenesis in lethal and opportunistic infections. *Ann. Surg.* 184:672-678, 1976.
137. Lanser, M.E., Saba, T.M., Scovill, W.A.: Opsonic glycoprotein (plasma fibronectin) levels after burn injury: Relationship to extent of burn and development of sepsis. *Ann. Surg.* 192:776-782, 1980.

138. Saba, T.M., Jaffe, E.: Plasma fibronectin (opsonic glycoprotein): its synthesis by vascular endothelial cells and role in cardiopulmonary integrity after trauma as related to reticuloendothelial function. *Am. J. Med.* 68:577-594, 1980.
139. Saba, T.M.: Prevention of liver reticuloendothelial systemic host defense failure after surgery by intravenous opsonic glycoprotein therapy. *Ann. Surg.* 188:142-152, 1978.
140. Lanser, M.E., Saba, T.M.: Correction of serum opsonic defects after burn and sepsis by opsonic fibronectin administration. *Arch. Surg.* 118:338-342, 1983.
141. Claret, I., Morales, L., Montaner, A.: Immunological studies in the postsplenectomy syndrome. *J. Ped. Surg.* 10:59-64, 1975.
142. Ellis, E.F., Smith, R.T.: The role of the spleen in immunity with special reference to the postsplenectomy problem in infants. *Pediatrics* 37:111-119, 1966.
143. Likhite, V.V.: Immunological impairment and susceptibility to infection after splenectomy. *JAMA* 236:1376-1377, 1976.
144. Rowley, D.A.: The formation of circulating antibody in the splenectomized human being following intravenous injection of heterologous erythrocytes. *J. Immunol.* 65:515-521, 1950.
145. Schulkind, M.L., Ellis, E.F., Smith, R.T.: Effect of antibody upon clearance of ¹²⁵I-labelled pneumococci by the spleen and liver. *Pediat. Res.* 1:178-184, 1967.
146. King, H., Shumacker, H.B.: Splenic studies. I. Susceptibility to infection after splenectomy performed in infancy. *Ann. Surg.* 136: 239-242, 1952.
147. Singer, D.B.: Postsplenectomy sepsis. *Perspect. Pediat. Path.* 1:285-311, 1973.
148. Chaudry, I.H., Tabata, Y., Schleck, S., Baue, A.E.: Effect of splenectomy on reticuloendothelial function and survival following sepsis. *J. Trauma* 20:649-656, 1980.
149. Scher, K.S., Wroczynski, F., Coil, J.A.: The effect of splenectomy on gram-negative bacteremia. *J. Trauma* 22:407-409, 1982.
150. Gullstrand, P., Alwmark, A., Schalén, C., Christensen, P.: Overwhelming pneumococcal infection in rats immediately after splenectomy. *J. Surg. Res.* 33:220-224, 1982.
151. Hunt, D.R., Allison, M.E.M., Prentice, C.R.M., Blumgart, L.H.: Endotoxemia, disturbances of coagulation, and obstructive jaundice. *Am. J. Surg.* 144:325-329, 1982.

152. Cahill, C.J.: Prevention of postoperative renal failure in patients with obstructive jaundice - the role of bile salts. *Br. J. Surg.* 70: 590-595, 1983.
153. Halpern, B.N., Biozzi, G., Nicol, T., Bilbey, D.L.J.: Effect of experimental biliary obstruction on the phagocytic activity of the reticulo-endothelial system. *Nature* 180:503-504, 1957.
154. Lásár, G.: Influence of splenectomy, partial hepatectomy and bile duct ligation on the granulopoietic activity of the reticuloendothelial system. *Acta Physiol. Acad. Sci. Hung.* 42:287-291, 1972.
155. Holman, J.M., Rikkers, L.: Biliary obstruction and host defense failure. *J. Surg. Res.* 32:208-213, 1982.
156. Iio, M., Wagner, H.N.: Studies of the reticuloendothelial system (RES). I. Measurement of the phagocytic capacity of the RES in man and dog. *J. Clin. Invest.* 42:417-426, 1963.
157. Drivas, G., James, O., Wardle, N.: Study of reticuloendothelial phagocytic capacity in patients with cholestasis. *Br. Med. J.* 1:1568-1569, 1976.
158. Wardle, N., Anderson, A., James, O.: Kupffer cell phagocytosis in relation to BSP clearance in liver and inflammatory bowel diseases. *Dig. Dis. Sci.* 25:414-419, 1980.
159. Katz, S., Grosfeld, J.L., Gross, K., Plager, D.A., Ross, D., Rosentahl, R.S., Hull, M., Weber, T.R.: Impaired bacterial clearance and trapping in obstructive jaundice. *Ann. Surg.* 199:14-20, 1984.
160. Cameron, G.R., Oakely, C.L.: Ligation of the common bile duct. *J. Path. Bact.* 35:769-798, 1932.
161. Ronai, P.M., Magarey, F.R.: Experimental cholangitis following biliary obstruction. *Australasian Ann. Med.* 9:289-294, 1960.
162. Cameron, G.R., Hasan, M.: Disturbances of structure and function in the liver as the result of biliary obstruction. *J. Path. Bact.* 75: 333-349, 1958.
163. Brodie, B.C.: On the effects produced by the bile in the process of digestion. *Quart. J. Sci.* 14:341-346, 1823.
164. Legg, J.W.: On the changes in the liver which follow ligation of the bile ducts. *St. Barth's Hosp. Rep.* 9:161-181, 1873.
165. Charcot, L.M., Gombault, A.: Note sur les altérations du foie consécutives à la ligation du canal cholédoque. *Arch. Physiol. norm. path.* 3:272-299, 1876.
166. Weinbren, K.: The effect of bile duct obstruction on regeneration of the rat's liver. *Br. J. exp. path.* 34:280-289, 1953.

167. Cameron, G.R., Prasad, L.B.M.: Recovery from biliary obstruction after spontaneous restoration of the obstructed common bile duct. *J. Path. Bact.* 80:127-136, 1960.
168. Steiner, P.E., Martinez, J.B.: Effects on the rat liver of bile duct, portal vein and hepatic artery ligations. *Am. J. Path.* 39:13-28, 1961.
169. Wright, J.E., Braithwaite, J.L.: The effects of ligation of the common bile duct in the rat. *J. Anat.* 98:227-233, 1964.
170. Freedman, L.R.: The effect of common bile duct obstruction on susceptibility of the liver to bacterial infection. *Yale J. Biol. Med.* 35:301-317, 1963.
171. Chou, S.T., Gibson, J.B.: Experimental cholangitis and cholelithiasis. *Br. J. exp. path.* 49:565-573, 1968.
172. Wichterman, K.A., Baue, A.E., Chaudry, I.H.: Sepsis and septic shock - A review of laboratory models and a proposal. *J. Surg. Res.* 29:189-201, 1980.
173. Alwmark, A., Bengmark, S., Gullstrand, P., Schalén, C.: Improvement of the splenectomized rat model for overwhelming pneumococcal infection. *Eur. surg. Res.* 13:339-343, 1981.
174. McConahey, P.J., Dixon, F.J.: A method of trace iodination of proteins for immunologic studies. *Int. Arch. Allergy* 29:185-189, 1966.
175. Persson, R.B.R., Naversten, Y.: Technetium-99m sulfide colloid preparation for scintigraphy of the reticuloendothelial system. *Acta Radiol. Ther. Phys. Biol.* 9:567-576, 1970.
176. Bergqvist, L., Strand, S.E., Persson, B.: Particle sizing and biokinetics of interstitial lymphoscintigraphic agents. *Semin. Nucl. Med.* 12:9-19, 1983.
177. Persson, R.B.R., Strand, S.E., Knöös, T.: Radioanalytical studies of ^{99m}Tc-labelled colloids and macromolecules with gel chromatography column scanning technique. *J. Radioanal. chem.* 43:275-286, 1978.
178. Medgyesi, G.A., Füst, G., Gergely, J., Bazin, H.: Classes and subclasses of rat immunoglobulins: Interaction with the complement system and with staphylococcal protein A. *Immunochemistry* 15:125-129, 1978.
179. Rydén, S., Bergqvist, L., Hafström, L., Hultberg, B., Stenram, U., Strand, S.E.: Influence of a reticuloendothelial suppressing agent on liver tumor growth in the rat. *J. Surg. Oncol.* 26:245-251, 1984.
180. Hampton, J.C.: An electron microscope study of the hepatic uptake and excretion of submicroscopic particles injected into the blood stream and into the bile duct. *Acta Anat.* 32:262-291, 1958.

181. Steiner, J.W., Carruthers, J.S., Kallait, S.R.: Disturbance of hydration of cells of rat liver in extrahepatic cholestasis. *Virchows Arch. Pathol. Anat. physiol. Klin. Med.* 336:99-114, 1962.
182. Tanikawa, K.: Ultra-structural aspects of the liver and its disorders. pp. 110-119, Springer-Verlag, New York, 1968.
183. Clark, A.G., Hirom, P.C., Millburn, P., Smith, R.L.: Absorption of some organic compounds from the biliary system of the rat. *J. Pharm. Pharmacol.* 23:150-152, 1971.
184. Peterson, R.E., Fujimoto, J.M.: Retrograde intrabiliary injection: Absorption of water and other compounds from the rat biliary tree. *J. Pharmacol. Exp. Ther.* 185:150-162, 1973.
185. Barber-Riley, G.: Measurement of capacity of biliary tree in rats. *Am. J. Physiol.* 205:1122-1126, 1963.
186. Olson, J.R., Fujimoto, J.M.: Demonstration of a D-glucose transport system in the biliary tree of the rat by use of the segmented retrograde intrabiliary injection technique. *Biochem. Pharmacol.* 29:213-219, 1980.
187. Hunter, W.M., Greenwood, F.C.: Preparation of Iodine-131 labelled human growth hormone of high specific activity. *Nature* 194:495-496, 1962.
188. Greenwood, F.C., Hunter, W.M., Glover, J.S.: The preparation of ¹³¹I-labelled human growth hormone of high specific radioactivity. *Biochem. J.* 89:114-123, 1963.
189. Ada, G.L., Nossal, J.V., Pye, J.: Antigens in immunity. III. Distribution of iodinated antigens following injection into rats via the hind footpads. *Aust. J. exp Biol. Med. Sci.* 42:295-310, 1964.
190. Thomas, H.C., Singer, C.R.J., Tilney, N.L., Folch, H., MacSween, R.N.M.: The immune response in cirrhotic rats. Antigen distribution, humoral immunity, cell-mediated immunity and splenic suppressor cell activity. *Clin. Exp. Immunol.* 26:574-582, 1976.
191. Marchalonis, J.J.: An enzymic method for the trace iodination of immunoglobulins and other proteins. *Biochem. J.* 113:299-305, 1969.
192. Saba, T.M., Antikatzides, T.G.: Heparin induced alterations in clearance and distribution of blood-borne microparticles following operative trauma. *Ann. Surg.* 189:426-432, 1979.
193. Rydén, S.: Reticuloendothelial function. Methods to evaluate function and correlation between function and tumor growth - experimental studies in the rat. Thesis, University of Lund, 1983.

194. Benacerraf, B., Biozzi, G., Halpern, B.N., Stiffel, C.: Physiology of phagocytosis of particles by the RES. In Halpern, B.N. (ed.): Physiopathology of the reticuloendothelial system. C.C. Thomas, Springfield, Ill., pp. 52-79, 1957.
195. Pirttiaho, H.I., Pitkänen, U.: Size and blood flow of the liver estimated by $^{99}\text{Tc}^{\text{m}}$ scanning. *Acta Radiol. Ther. Phys. Biol.* 16:497-506, 1977.
196. Atkins, H.L., Hauser, W., Richards, P.: Factors affecting distribution of Technetium-sulfur colloid. *J. Reticuloendo. Soc.* 8:176-184, 1970.
197. Boyd, G.S., Eastwood, M.A., MacLean, N.: Bile acids in the rat: studies in experimental occlusion of the bile duct. *J. lipid res.* 7: 83-94, 1966.
198. Greim, H., Trülzsch, D., Roboz, J., Dressler, K., Czygan, P., Hutterer, F., Schaffner, F., Popper, H.: Mechanisms of cholestasis. 5. Bile acids in normal rat livers and in those after bile duct ligation. *Gastroenterology* 63:837-850, 1972.
199. Newberry, W.M., Shovey, J.W., Sanford, J.P., Combes, B.: Depression of lymphocyte reactivity to phytohemagglutinin by serum from patients with liver disease. *Cell. Immunol.* 6:87-97, 1973.
200. Gianni, L., Padova, F.D., Zuin, M., Podda, M.: Bile acid-induced inhibition of the lymphoproliferative response to phytohemagglutinin and pokeweed nitrogen: An in vitro study. *Gastroenterology* 78:231-235, 1980.
201. Keane, R.M., Gadacz, T.R., Munster, A.M., Birmingham, W., Winchurch, R.A.: Impairment of human lymphocyte function by bile salts. *Surgery* 95:439-443, 1984.
202. Aronsen, K.F.: Liver function studies during and after complete extra-hepatic biliary obstruction in the dog. *Acta chir. scand.* suppl 275:1-114, 1961.
203. Schaffner, F., Popper, H.: Morphologic studies of cholestasis. *Gastroenterology* 37:565-573, 1959.
204. Sasaki, H., Schaffner, F., Popper, H.: Bile ductules in cholestasis: Morphologic evidence for secretion and absorption in man. *Lab. Invest.* 16:84-95, 1967.
205. Grün, M., Brotsch, Ch.E., Wolter, J.: Influence of portal hepatic blood flow on RES function. In: *The Reticuloendothelial System and the Pathogenesis of Liver Disease*. Elsevier, Amsterdam, pp. 149-158, 1980.

206. Aronsen, K.F., Nylander, G., Ohlsson, E.G.: Liver blood flow studies during and after various periods of total biliary obstruction in the dog. *Acta chir. scand.* 135:55-59, 1969.
207. Normann, S.J.: Kinetics of phagocytosis II: analysis of in vivo clearance with demonstration of competitive inhibition between similar and dissimilar foreign particles. *Lab. Invest.* 31:161-169, 1974.
208. Howard, J.G.: The reticulo-endothelial system and resistance to bacterial infection. *Scot. med. J.* 6:60-82, 1961.
209. Wood, W.B., Smith, M.R., Perry, W.D., Berry, J.W.: Studies on the cellular immunology of acute bacteremia. I. Intravascular leukocytic reaction and surface phagocytosis. *J. Exptl. Med.* 94:521-534, 1951.
210. Lanser, M.E., Saba, T.M.: Neutrophil-mediated lung localization of bacteria: A mechanism for pulmonary injury. *Surgery* 90:473-481, 1981.
211. Makuuchi, M., Bandai, Y., Ito, T., Watanabe, G., Wada, T., Abe, H., Muroi, T.: Ultrasonically guided percutaneous transhepatic bile drainage. A single step procedure without cholangiography. *Radiology* 136:165-169, 1980.

A RAT MODEL FOR SEPSIS IN CHRONIC BILIARY OBSTRUCTION

Nobutaka Tanaka¹, Poul Christensen², Stefan Rydén¹, Britten Klöfver-Ståhl¹, Stig Bengmark¹

¹Department of Surgery and ²Department of Medical Microbiology, University of Lund, S-221 85 LUND, Sweden

Running title: Rat sepsis in biliary obstruction

Abstract

The effect of retrograde intrabiliary (RI) injection of *E. coli* was studied in Sprague-Dawley rats with and without chronic biliary obstruction. The challenge dose of *E. coli* was standardized by the use of frozen (-80°C) aliquots of bacteria. Injection of 10^2 , 10^5 or 10^8 colony forming units (CFU), respectively, immediately after occlusion of the common bile duct (CBD) into three groups of 8 normal rats did not kill any of the animals. In contrast, 5 of 8 animals with chronic biliary obstruction died from *E. coli* sepsis after RI injection of 10^2 bacteria ($p < 0.05$). Furthermore, all of 4 obstructed animals died after challenge with 10^5 CFU ($p < 0.01$ as compared to the 8 normal rats surviving this dose). Intraperitoneal injection of 10^5 *E. coli* did not kill any of 6 animals with 3 weeks' biliary obstruction. It is concluded that chronic biliary obstruction and RI injection are prerequisites for the occurrence of lethal septicaemia in the animals. The model might be suitable for the study of biliary sepsis in chronic biliary obstruction.

Key words: Biliary obstruction, septicaemia, *E. coli*.

Nobutaka Tanaka, Department of Surgery, University of Lund, S-221 85 Lund, Sweden.

Sepsis is a major threat to patients with chronic biliary obstruction (8, 14, 17) or acute suppurative cholangitis (4, 10, 19). The mortality rates range from 9 to 40 % (22). In order to clarify the mechanism(s) leading to sepsis in these underlying diseases, and to improve treatment regimens, there are persisting demands for establishing animal models for biliary sepsis. Several workers have tried intravenous inoculation of the bacteria, although a haematogenous spread of bacteria to the biliary system is highly speculative (5, 6, 9, 20). These studies invariably demonstrated an increased susceptibility of biliary obstructed animals to bacterial challenge.

One mechanism behind biliary sepsis might be an ascending invasion of bacteria from the intestine. To our knowledge, no experimental model of fulminant septicaemia originating from the biliary tree has so far been described. Jacobson et al. (15) and Huang et al. (13) injected bacteria into the biliary tree of dogs to investigate the relationship between regurgitation of bacteria and intraductal pressure, but these experiments did not involve e.g., clinical pictures or survival studies. The present paper concerned a model in which a standardized inoculum of *E. coli* was injected directly into the biliary tree of rats with chronic biliary obstruction. These animals proved highly susceptible to the bacterial challenge.

MATERIALS AND METHODS

Animals

Fifty Sprague-Dawley rats of both sexes weighing 220-340 g were used.

Bacteria

An *E. coli* strain was isolated at our routine bacteriological laboratory and frozen in aliquots at -80°C , as previously described by our group for pneumococci (1). Immediately before use, the bacteria were diluted in Todd Hewitt broth to obtain 0.1 ml volumes containing 10^8 , 10^5 or 10^2 colony forming units (CFU), respectively.

Surgical procedures and injection of bacteria

The effect of bacterial challenge was studied in 32 normal rats and in 18 rats in which the common bile duct (CBD) had been obstructed for 21 days. Extrahepatic obstruction of CBD was created by double ligation and excision of 5 - 8 mm of the middle part of the CBD, in order to prevent recanalization, as described by Trams and Symeonidis (23).

Twenty-four of the normal rats and 12 of the rats with biliary obstruction were given retrograde intrabiliary (RI) injections of bacteria. The remaining animals received the bacteria intraperitoneally (IP). For RI injections in the normal rats, the CBD was isolated and ligated (6-0 silk) just above the entrance of the highest branch of the pancreatic duct. Immediately after ligation, 0.1 ml bacteria were injected proximally above the ligature with a fine hypodermic needle (0.2 mm diameter). In order to minimize leakage of bacteria to the intraperitoneal space, the inserted

needle was tied to the bile duct by another thread. This thread was used to ligate the proximal CBD just above the needle hole after withdrawal of the needle. In obstructed rats, a purse string suture (6-0 silk) was applied to the dilated CBD to prevent leakage of the bacteria. Immediately before injection of bacteria, 0.1 ml of bile was withdrawn for culture. Two of 14 rats with chronic biliary obstruction were subsequently excluded from the present study due to the infected bile, although they were found resistant to bacterial challenge probably owing to the previous immunization. Care was taken to inject bacteria very slowly (usually, the injection took at least 40 seconds) with a minimum of pressure. No substantial caliber change in CBD was seen during injection. All surgical procedures, including RI and IP injections, were performed under ether anaesthesia, using sterile conditions. After challenge, the animals were kept under standard laboratory conditions with food (pellets) and water *ad libitum* and observed every sixth hour for 7 days. Succumbed rats were autopsied, and 0.1 ml heart blood and bile was spread on blood agar plates. After overnight incubation at 37 °C, randomly selected colonies were identified using routine fermentation reaction. Surviving rats were sacrificed 7 days after challenge and samples were taken for culture from heart blood and bile.

Statistical method

Survival rates were evaluated using Fischer's exact (probability) test.

RESULTS

After RI injections of *E. coli* in doses ranging from 10^2 to 10^8 CFU, all 24 normal rats survived without clinical signs of infection. In contrast, all of the 8 rats with chronic biliary obstruction became ill with tachypnea, piloerection and reduced intake of food and water after injection of 10^2 *E. coli*; 5 of these rats died within 3 days (median survival time 36 hrs, range 23 - 54 hrs). Thus, the jaundiced rats were highly susceptible to *E. coli* as compared to all 24 normal rats (< 0.01) or to each of the three groups of 8 normal rats shown in Table I ($p = 0.01$). The sus-

TABLE I. Outcome of bacterial challenge of normal rats and animals with chronic biliary obstruction.

Groups of animals	Challenge dose of <i>E. coli</i>	Route of challenge	Number of animals	Number of killed animals
Normal rats	10^2	RI ¹	8	0
	10^3	RI	8	0
	10^6	RI	8	0
	10^5	IP ²	8	0
Rats with 21 days' chronic biliary obstruction	10^2	RI	8	5*
	10^5	IP	6	0

¹RI: retrograde intrabiliary injection

²IP: intraperitoneal injection

*Significantly more animals died in this group as compared to each group of normal rats ($p = 0.0128$) or the IP injected animals ($p = 0.0300$)

ceptibility of the rats with CBD obstruction was confirmed in a subsequent experiment, in which all of 4 animals with jaundice died after injection of 10^5 *E. coli* ($p < 0.01$ as compared to the 8 normal rats surviving the same dose).

IP injection of 10^5 *E. coli* did not kill any of 8 normal rats or any of 6 rats with chronic biliary obstruction.

Blood and bile cultures from all succumbed rats grew *E. coli*. In normal rats, positive cultures were obtained only from the rats receiving 10^8 *E. coli* by RI injection. Thus, the blood culture grew *E. coli* in one animal and 7 of 8 rats showed bile cultures positive for *E. coli*. These animals also showed pus formation confined to the biliary tree. In the animals with chronic biliary obstruction, 2 of 3 rats surviving the 10^2 *E. coli* challenge showed positive bile cultures. Blood cultures were negative in all chronically obstructed rats surviving RI and IP challenge.

DISCUSSION

Normal rats were found to be highly resistant to RI injection of *E. coli*, even in as high a dose as 10^8 CFU. IP injection of 10^5 *E. coli* did not kill normal animals or rats with chronic obstruction of the CBD. In contrast, rats with chronic biliary obstruction were markedly susceptible to RI injection, i.e., 5 of 8 rats died from 10^2 *E. coli* (Table I). These results were corroborated by the finding that all of 4 rats with chronic jaundice died by RI injection of 10^5 *E. coli*. Finally, blood cultures were positive for *E. coli* in all succumbed rats, whereas such samples were negative in 40/41 surviving animals. Taken together, the results clearly indicated that both the presence of extrahepatic biliary obstruction and the introduction of bacteria into the biliary system were prerequisites for production of sepsis. These findings increased the credibility of the model for biliary sepsis. Studies are in progress to elucidate whether other *E. coli* strains than the randomly selected strain used in the present experiments might have a similar effect.

In order to avoid the possibility that the liver and the bile could be colonized in the obstructed animals before the RI injection, rats with infected bile were excluded from the study. Ligation of the CBD in connection with injection of the bacteria was performed to prevent leakage of bile and bacteria to the peritoneum. Furthermore, if the CBD is not ligated, the injected bacteria might flow down to the intestines so that standardization of the bacterial dose might be difficult to achieve. Preliminary studies using ^{125}I -labeled *E. coli* have shown that 30 - 40 % of the injected dose could be recovered in the bile within 15 min after RI injection if no ligation was performed. Thus, only 60 - 70 % was supposed to reach the hepatobiliary system (Tanaka, unpublished observation).

RI injection of particles in small animals has also been utilized in studies of the jaundice process (14, 21), liver micropathology (3), and absorption of several compounds from the biliary tree (18). With the exception of the elaborate work by Peterson and Fujimoto (18), it might be suspected that the injection volumes have been too large to maintain the normal integrity of the biliary tree. In the present study, no more than

0.1 ml bacterial suspension was injected into the biliary system in order to avoid distension (2).

The mechanism behind the increased susceptibility to *E. coli* after a long-standing CBD obstruction is at present not fully understood. Several authors have referred to impaired *Kupffer cell* phagocytosis to explain the increased frequency of sepsis and the impaired bacterial clearance in chronic biliary obstruction (7, 12, 16). A defective local immune system in the biliary tree might also play a role in the present model.

In conclusion, RI injection of *E. coli* demonstrated an increased susceptibility to bacteria in rats with chronic biliary obstruction. This model may facilitate further studies on mechanisms of biliary sepsis.

REFERENCES

1. Alwmark, A., Bengmark, S., Gullstrand, P. & Schalén, C.: Improvement of the splenectomized rat model for overwhelming pneumococcal infection. *European surgical Research*. 13: 339-343, 1981.
2. Barber-Riley, G.: Rat biliary tree during short periods of obstruction of common bile duct. *American Journal of Physiology*. 205: 1127-1131, 1963.
3. Bockman, D.E.: Route of flow and micropathology resulting from retrograde intrabiliary injection of India ink and ferritin in experimental animals. *Gastroenterology*. 67: 324-332, 1974.
4. Boey, J.H. & Way, L.W.: Acute cholangitis. *Annals of Surgery*. 191: 264-270, 1980.
5. Chou, S.T. & Gibson, J.B.: Experimental cholangitis and cholelithiasis. *British Journal of Experimental Pathology*. 49: 565-573, 1968.
6. Dineen, P.: The importance of the route of infections in experimental biliary tract obstruction. *Surgery, Gynecology & Obstetrics*. 119: 1001-1008, 1964.
7. Drivas, G., James, O. & Wardle, N.: Study of reticuloendothelial phagocytic capacity in patients with cholestasis. *British Medical Journal*. 1: 1568-1569, 1976.
8. Flemma, R.J., Flint, L.M., Osterhout, S. & Shingleton, W.W.: Bacteriologic studies of biliary tract infection. *Annals of Surgery*. 166: 563-572, 1967.
9. Freedman, L.R.: The effect of common bile duct obstruction on susceptibility of the liver to bacterial infection. *Yale Journal of Biology and Medicine*. 35: 301-317, 1963.
10. Glenn, F. & Moody, F.G.: Acute obstructive suppurative cholangitis. *Surgery, Gynecology & Obstetrics*. 113: 265-273, 1961.
11. Hampton, J.C.: Electron microscopic study of extrahepatic biliary obstruction in the mouse. *Laboratory Investigation*. 10: 502-513, 1961.
12. Holman, J.M. & Rikkers, L.: Biliary obstruction and host defense failure. *Journal of Surgical Research*. 32: 208-213, 1982.
13. Huang, T., Bass, J.A. & Williams, R.D.: The significance of biliary pressure in cholangitis. *Archives of Surgery*. 98: 629-632, 1969.
14. Jackaman, F.R., Hilson, G.R.F. & Smith, M.: Bile bacteria in patients with benign bile duct stricture. *British Journal of Surgery*. 67: 329-332, 1980.

15. Jacobson, B., Kjellander, J. & Rosengren, B.: Cholangiovenous reflux. *Acta Chirurgica Scandinavica*. 123: 316-321, 1962.
16. Katz, S., Gorsfeld, J.L., Gross, K., Player, D.A., Ross, D., Rosenthal, R.S., Hull, M. & Weber, T.R.: Impaired bacterial clearance and trapping in obstructive jaundice. *Annals of Surgery*. 199: 14-20, 1984.
17. Ong, G.B.: A study of recurrent pyogenic cholangitis. *Archives of Surgery*. 84: 199-225, 1962.
18. Peterson, R.E. & Fujimoto, J.M.: Retrograde intrabiliary injection: Absorption of water and other compounds from the rat biliary tree. *Journal of Pharmacology and Experimental Therapeutics*. 185: 150-162, 1973.
19. Reynolds, B.M. & Dargan, E.L.: Acute obstructive cholangitis. *Annals of Surgery*. 150: 299-303, 1959.
20. Ronai, P.M. & Magarey, F.R.: Experimental cholangitis following biliary obstruction. *Australasian Annals of Medicine*. 9: 289-294, 1960.
21. Steiner, J.W., Carruthers, J.S. & Kalifat, R.: Disturbances of hydration of cells of rat liver in extrahepatic cholestasis. *Virchow's Archives of Pathology and Anatomy*. 336: 99-114, 1962.
22. Thompson, J.E., Thompkins, R.K. & Longmire, W.P.: Factors in management of acute cholangitis. *Annals of Surgery*. 195: 137-145, 1982.
23. Trams, E.G. & Symeonidis, A.: Morphologic and functional changes in the livers of rats after ligation or excision of the common bile duct. *American Journal of Pathology*. 33: 13-27, 1957.

BILIARY OBSTRUCTION AND SUSCEPTIBILITY TO BILIARY SEPSIS IN RATS

N. Tanaka^a, P. Christensen^b, S. Rydén^a, B. Klöfver-Ståhl^a, S. Bengmark^a

^aDepartment of Surgery and ^bDepartment of Microbiology, University of Lund, Sweden

Running head: Biliary sepsis in biliary obstruction

Key words: Biliary obstruction - Biliary sepsis - Cholangitis - Intra-biliary injection - E. coli - Rat

Abstract

The effect of retrograde intrabiliary (RI) injection of E. coli was studied in Sprague-Dawley rats with biliary obstruction of different durations (i.e., 3 days, 2 weeks, 4 weeks and 6 weeks). By the injection of 10^5 colony forming units (CFU) immediately after occlusion of the common bile duct (CBD), 15 of 18 normal rats survived without clinical signs of infection. In contrast, 6 of 11 animals in 3 days' obstruction ($p = 0.04$), 7 of 12 in 2 weeks' obstruction ($p = 0.02$), 10 of 12 in both 4 weeks' and 6 weeks' obstruction ($p = 0.0004$) died from E. coli sepsis after injection of the same amount of bacteria. Animals with long-standing jaundice (4 and 6 weeks) were more susceptible than those with shorter duration of jaundice (3 days and 2 weeks) ($p = 0.04$). The results warrant the early decompression of the biliary tract in biliary obstruction.

INTRODUCTION

Biliary obstruction predisposes to cholangitis and sepsis in experimental animals as well as humans (6, 9, 11, 15, 20, 23). The mechanism leading to increased susceptibility to infection has not yet been defined, but impairment of the reticuloendothelial system (RES) of the liver has been suggested to play an important role (8, 14, 16).

After occlusion of the bile duct in experimental animals, the deterioration of the liver function progresses with time (2, 3, 25). So far, the relation between susceptibility to bacteria and the duration of biliary obstruction has not been clearly demonstrated. Studies on this relation are expected to elucidate the pathogenesis of biliary sepsis and also influence the clinical management of patients with biliary obstruction.

We have previously developed a rat model of fulminant septicaemia originating in the biliary tree (24). Rats with 3 weeks' biliary obstruction were demonstrated to be highly susceptible to a standardized retrograde intrabiliary (RI) injection of E. coli, whereas the same bacterial challenge immediately after occlusion had no effect. The aim of the present paper is to study the effect of the duration of the biliary obstruction on the susceptibility to this standardized E. coli challenge.

MATERIALS AND METHODS

Animals. Sixty-five male Sprague-Dawley rats initially weighing 250-280 g were used.

Surgical procedures and injection of bacteria. Extrahepatic biliary obstruction was created by double ligation of the common bile duct (CBD) and excision of 5 - 8 mm of the middle part of the duct between the ligatures to prevent recanalization (25). The 47 rats with biliary obstruction were divided into 4 groups according to the duration of obstruction: Group I, 3 days (n = 11); Group II, 2 weeks (n = 12); Group III, 4 weeks (n = 12); and Group IV, 6 weeks (n = 12).

For bacterial challenge, an E. coli strain was frozen in aliquots at -80 °C (1, 24). The bacteria were thawed immediately before use and diluted in Todd Hewitt broth to obtain 0.1 ml volumes containing 10^7 colony forming units (CFU). RI injections of the bacteria were performed as previously described (24). In brief, in normal rats (n = 18), slow injection of 0.1 ml bacteria took place proximally above a ligature applied on the CBD just above the entrance of the highest branch of the pancreatic duct. After injection, the CBD was again ligated proximally to the needle hole with another thread to prevent leakage of bacteria. In the rats with CBD obstruction, a purse-string suture applied on the dilated bile duct prevented leakage of bacteria after injection.

Surgical procedures including RI injections were performed under ether anaesthesia using clean but not sterile conditions. After challenge, the animals were kept under standard laboratory conditions with food and water ad libitum, and observed every sixth hour for 7 days.

Surviving rats were sacrificed 7 days after the injection. Succumbed and surviving rats were autopsied and cultures from heart blood and bile of all animals were collected and processed as previously described (24).

Statistical method. Survival rates were evaluated using Fisher's exact (probability) test.

RESULTS

At the time of injection, all the rats with CBD obstruction showed a more or less dilated CBD, the diameter being proportionally larger with the duration of the obstruction. The rats with more than 4 weeks' jaundice invariably showed a pronounced cystic dilatation of the bile duct.

After RI injection of E. coli, 15 of 18 normal rats survived without clinical signs of infection, whereas 3 controls died in haemorrhagic peritonitis. In contrast, all of the rats with obstructive jaundice showed tachypnoea, piloerection and reduced intake of food and water immediately after challenge. Among the rats with 3 days of jaundice, 6 of 11 died within 2 days after injection (median survival time in the succumbed animals was 23 h, range 18 - 40 h). In the group of rats with 2 weeks' obstruction, 7 of 12 died within 3 days (median survival time in the succumbed animals was 23 h, range 19 - 56 h). In the remaining two groups with 4 and 6 weeks' obstruction, 10 of 12 animals died in both groups (median survival times, 40 h and 44 h, ranges 20 - 84 and 12 - 56 h, respectively).

Table I. Susceptibility of rats with biliary obstruction to retrograde intrabiliary injection¹ of E. coli².

Period of biliary obstruction (Group)	Number of animals challenged	Number (per cent) of animals succumbed	p-value as compared to controls ³
3 days (I)	11	6 (55)	0.04
2 weeks (II)	12	7 (58)	0.02
4 weeks (III)	12	10 (83)	0.0004
6 weeks (IV)	12	10 (83)	0.0004
Normal rats	18	3 (17)	-

¹ see Materials and Methods for procedure

² 10^5 colony forming units

³ I + II compared to III + IV was significantly different ($p = 0.04$)

Thus, the jaundiced rats in all 4 groups were significantly more susceptible to RI injection of E. coli than the controls (Table I). On the other hand, there were no significant differences between any of the four groups with biliary obstruction. When the two early groups (3 days' and 2

weeks' jaundice) were combined and compared to the animals with more long-standing jaundice (4 and 6 weeks) a significant difference in the susceptibility to E. coli injection was observed: 13/23 died in the former whereas 20/24 died in the latter (Table I).

Blood and bile cultures from all succumbed rats grew E. coli. In contrast, blood cultures were negative in all the rats surviving RI challenge. Bile cultures from surviving animals with biliary obstruction were all positive, while half of the normal rats surviving after challenge showed positive bile culture.

DISCUSSION

The present study demonstrated that the mortality caused by RI injection of E. coli increased already three days after obstruction of the CBD in rats. The susceptibility to biliary sepsis was also found to increase with the duration of the obstruction.

Ronai and Magarey in 1960 (20) and Chou and Gibson in 1968 (6) injected coliform bacteria into the portal vein of rats and obtained a high mortality rate immediately after obstruction of the CBD. The apparent discrepancy to the present results might well be explained by the different routes of bacterial challenge. Our model tested the capacity of the local immune system of the liver to prevent spreading of the bacteria to the blood stream whereas this barrier was circumvented in earlier studies.

RES phagocytic function measured by plasma disappearance rate and organ localization of particles (12, 14, 18) or radiolabelled bacteria (16) was reported to be intact at least until three weeks after biliary obstruction in the rat. The present finding that the animals became susceptible to bacteria already three days after biliary obstruction might therefore be ascribed to other mechanisms than phagocytosis. Phagocytosis is associated with an increased consumption of oxygen, followed by production of oxygen metabolites with antibacterial effects, e.g., hydrogen peroxide (21). One possible explanation to the early susceptibility to E. coli following biliary obstruction could be deficiencies in this system like in chronic granulomatous disease (17).

Another mechanism behind the early susceptibility to bacteria might be related to the regurgitation of bacteria into the systemic circulation. Bile constituents are known to appear in the blood soon after biliary obstruction. This phenomenon has been ascribed to changes of either hepatocyte secretion (13), canalicular membrane (22), or tight junctions (7, 19). Liver cell necrosis which occurs soon after biliary obstruction (4, 5) might also accelerate the regurgitation of bile. Whether bacteria introduced into the biliary tree could pass to the systemic circulation in animals with biliary obstruction needs some further studies.

The finding that the susceptibility to E. coli increased with the duration of the obstruction is consistent with the progressive impairment of the liver function seen in animals with long-standing obstructive jaundice (2). We conclude from the present investigation that early decompression of the biliary tract in obstruction of the CBD is warranted.

REFERENCES

1. Alwmark, A.; Bengmark, S.; Gullstrand, P.; Schalén, C.: Improvement of the splenectomized rat model for overwhelming pneumococcal infection. *Eur. surg. Res.* 13: 339-343 (1981).
2. Aronsen, K.F.: Liver function studies during and after complete extrahepatic biliary obstruction in the dog. *Acta Chir. Scand. suppl.*, 275: 1-114 (1961).
3. Cameron, G.R.; Hasan, M.: Disturbances of structure and function in the liver as the result of biliary obstruction. *J. Path. Bact.* 75: 333-349 (1958).
4. Cameron, G.R.; Oakely, C.L.: Ligation of the common bile duct. *J. Path. Bact.* 35: 769-798 (1932).
5. Charcot; Gombault: Note sur les altérations du foie consécutives à la ligature du canal cholédoque. *Arch. phys. norm. path.* 8: 272-299 (1876).
6. Chou, S.T.; Gibson, J.B.: Experimental cholangitis and cholelithiasis. *Brit. J. exp. path.* 49: 565-573 (1968).
7. De Vos, R.; Desmet, V.J.: Morphologic changes of the junctional complex of the hepatocytes in rat liver after bile duct ligation. *Brit. J. exp. path.* 59:220-227 (1978).
8. Drivas, G.; James, O.; Wardle, N.: Study of reticuloendothelial phagocytic capacity in patients with cholestasis. *Br. Med. J.* 1: 1568-1569 (1976).
9. Flemma, R.J.; Flint, L.M.; Osterhout, S.; Shingleton, W.W.: Bacteriologic studies of biliary tract infection. *Ann. Surg.* 166: 563-572 (1967).
10. Freedman, L.R.: The effect of common bile duct obstruction on susceptibility of the liver to bacterial infection. *Yale J. Biol. Med.* 35: 301-317 (1963).
11. Freedman, L.R.: The relationship between bacterial infection of the hepato-biliary system and common bile duct obstruction in man. *Yale J. Biol. Med.* 35: 318-327 (1963).
12. Halpern, B.N.; Biozzi, G.; Nicol, T.; Bilbey, D.L.J.: Effect of experimental biliary obstruction on the phagocytic activity of the reticuloendothelial system. *Nature* 180: 503-504 (1957).
13. Hampton, J.C.: Electron microscopic study of extrahepatic biliary obstruction in the mouse. *Lab. Invest.* 10: 502-513 (1961).
14. Holman J.M.; Rikkers, L.F.: Biliary obstruction and host defense failure. *J Surg. Res.* 32: 208-213 (1982).

15. Jackaman, F.R.; Hilson, G.R.F.; Lord Smith of Marlow: Bile bacteria in patients with benign bile duct stricture. *Br. J. Surg.* 67: 329-332 (1980).
16. Katz, S.; Grosfeld, J.L.; Gross, K.; Plager, D.A.; Ross, D.; Rosenthal, R.S.; Hull, M.; Weber, T.R.: Impaired bacterial clearance and trapping in obstructive jaundice. *Ann. Surg.* 199: 14-20 (1984).
17. Klebanoff, S.J.: Iodination of bacteria: A bactericidal mechanism. *J. Exp. Med.* 126: 1063-1079 (1967).
18. Lázár, G.: Influence of splenectomy, partial hepatectomy and bile duct ligation on the granulopoietic activity of the reticuloendothelial system. *Acta Phy. Acad. Sci. Hung.* 42: 287-291 (1972).
19. Metz, J.; Aoki, A.; Merlo, M.; Forssmann, W.G.: Morphological alterations and functional changes of interhepatocellular junctions induced by bile duct ligation. *Cell Tiss. Res.* 182: 299-310 (1977).
20. Ronai, P.M.; Magarey, F.R.: Experimental cholangitis following biliary obstruction. *Australasian ann. med.* 9: 289-294 (1960).
21. Root, R.K.; Cohen, M.S.: The microbicidal mechanisms of human neutrophils and eosinophils. *Rev. Infect. Dis.* 3: 565-598 (1981).
22. Schaffner, F.; Popper, H.: Morphologic studies of cholestasis. *Gastroenterology* 37: 565-572 (1959).
23. Scott, A.J.; Jhan, G.A.: Origin of bacteria in bile duct bile. *Lancet* ii: 790-792 (1967).
24. Tanaka, N.; Christensen, P.; Rydén, S.; Ögren, M.; Klöfver-Ståhl, B.; Bengmark, S.: A rat model for sepsis in chronic biliary obstruction. *J. Surg. Res.*, submitted for publication.
25. Trams, E.G.; Symeonidis, A.: Morphologic and functional changes in the livers of rats after ligation or excision of the common bile duct. *Am. J. Path.* 33: 13-27 (1957).

DECREASED CLEARANCE OF ESCHERICHIA COLI FROM THE BILE IN RATS WITH
OBSTRUCTIVE JAUNDICE

N. Tanaka^a, P. Christensen^b, S. Rydén^a, B. Klöfver-Ståhl^a, S. Bengmark^a

^aDepartment of Surgery and ^bDepartment of Microbiology, University of
Lund, Sweden

Running head: Biliary clearance of bacteria in obstructive jaundice

Key words: Biliary obstruction - Radiolabelled E. coli - Biliary
clearance of bacteria - Biliary sepsis - Rat

Abstract

Clearance of E. coli from the bile was studied in 4 normal Sprague-Dawley rats and 4 rats with 3 weeks' obstruction of the common bile duct (CBD). ¹²⁵I radiolabelled heat-killed E. coli were injected into the CBD and the radioactivity of the bile was monitored for 2 hours. The radioactivity declined exponentially during the first 10 min. Bile samples collected from 2 min to 15 min after injection showed higher amounts of radioactivity in all rats with biliary obstruction than in the normal rats. However, the clearance rate was higher in normal than in obstructed rats ($p < 0.05$). It was therefore concluded that the bacteria were cleared off the bile rapidly in normal rats by the function of a liver and/or a biliary tree. The present data, concerning the kinetic study of bacterial clearance from the biliary tract, indicated that the impaired clearance of bacteria in chronic biliary obstruction might be crucial for the development of biliary sepsis.

INTRODUCTION

Biliary infections are frequently seen in patients with obstructive jaundice (3, 6, 11). Although it has been suggested that an impaired function of the reticuloendothelial system (RES) of the liver might play a major pathogenic role (2, 5, 8), the precise mechanism(s) leading to biliary sepsis in obstructive jaundice is not yet clear.

We have currently shown that rats with chronic biliary obstruction are highly susceptible to retrograde intrabiliary (RI) injection of *Escherichia coli* (*E. coli*) (12). Thus, introduction of 10^2 *E. coli* into the biliary tree in rats with 3 weeks' obstruction resulted in 63 per cent mortality whereas normal rats immediately after ligation of the common bile duct (CBD) survived 10^8 bacterial challenge. These results suggested the impaired capacity of the rats with chronic jaundice to cope with the ascending bacteria. On this background, it seemed interesting to study the ability of the liver to clear bacteria from the biliary tree. The present paper concerned a model in which radiolabelled *E. coli* was injected in the CBD of rats with and without biliary obstruction. Then the disappearance of bacteria from the bile was followed by monitoring the radioactivity of bile.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing 270-357 g were used. The animals were maintained on an artificial day and night cycle and fed laboratory food (pellets) and water ad libitum.

Radiolabelling of bacteria. An *E. coli* strain (12) was cultured overnight in 10 ml Todd Hewitt broth, heat-killed at 80°C for 30 min and washed three times in phosphate-buffered saline (PBS, 0.03 M phosphate, 0.12 M NaCl, pH 7.2) followed by suspension in 4 ml PBS. The bacteria were then labelled with ^{125}I according to the protein-labelling method of McConahey and Dixon (9). After three further washings in PBS, the bacteria were frozen in aliquots at -20°C and used within 2 weeks. The radioactivity was approximately 2×10^6 counts per minute (cpm)/ml bacterial suspension.

Surgical procedures and measurement of bile clearance of bacteria. Experiments were carried out under light ether anaesthesia with clean but not sterile conditions. The experiment in each rat was started at 8 a.m. exactly to standardize day rhythm effects on bile flow (4, 14). Rectal temperature was maintained between 37°C and 37.5°C .

Extrahepatic obstruction of the CBD was created by double ligation and excision of 5-8 mm of the middle part of the CBD to prevent recanalization (13).

RI injection of ^{125}I -labelled *E. coli* was performed in 4 normal rats and in 4 animals with biliary obstruction for 3 weeks. The CBD was cannulated with 10 cm of a silastic catheter (Dow Corning Corporation, Michigan, USA; 0.012 in. ID, 0.025 in. OD, volume approximately 9 μl) with the distal end temporarily occluded by a haemostatic clip until the injection of bacteria was finished. In the obstructed rats, the catheter inserted into

the cystically dilated CBD was secured with a purse-string suture, making efforts to minimize the leakage of bile and thereby the decrease in biliary pressure. In normal rats, the catheter was fixed with a ligature around the CBD. RI injection of bacteria (0.1 ml volumes) was performed between the ligature used for fixation and a second ligature threaded proximally on the CBD in both normal and jaundiced rats. Since rapid injection of the fluid with increased pressure induces a substantially reduced bile flow (1), great care was taken to inject the suspension very slowly using a minimal pressure. Immediately after injection, the catheter was declamped and the bile was allowed to drain freely for collection, at 1 min interval until 15 min and then 10 min interval till 120 min. After bile flow was determined (Table I), 10 μ l of bile samples were taken for the measurement of radioactivity for each interval. In order to replace bile loss, a 0.9 % NaCl solution was administered intravenously at a rate of 0.05 ml/min.

For collection of blood samples (0.1 ml volumes) the femoral vein was cannulated. Blood samples were taken simultaneously with bile samples.

The radioactivity of the samples was determined using a 1260 Multigamma counter (LKB, Stockholm, Sweden), with a background not exceeding 60 cpm. The clearance rate of bacteria from the biliary tree was calculated by the formula: $K = (\log C_5 - \log C_{10}) / (T_{10} - T_5)$, where C_5 and C_{10} represented the radioactivity of the bile at the time T_5 (5 min) and T_{10} (10 min), respectively, after injection of the bacteria.

Statistical method. Differences in body weight and bile flow between normal and jaundiced rats were evaluated using Student's t-test. Differences in bile radioactivity were evaluated using Mann-Whitney's rank sum test. A p-value < 0.05 was considered to be significant.

RESULTS

Three weeks' biliary obstructed rats were deeply jaundiced and the CBD in these animals were invariably cystically dilated. Consequently, bile volumes recovered during the initial 30 min bile drainage in jaundiced rats were twice more than those in normal rats (Table I).

Table I. Body weight and bile flow in normal and jaundiced rats.

		Body weight (g)	Bile flow (μl/min)		
			0 - 30 min	30 min - 1 h	1 h - 2 h
Normal rats	(4)	285±14.1	12.6±0.8	18.9±1.5	21.9±1.8
Jaundiced rats	(4)	347±3.8 ^a	38.1±4.9 ^a	32.9±2.4 ^a	24.7±0.6

^a p < 0.05 compared to normal rats. Values are given as mean $\pm$ SE. Numbers of rats in parentheses.

One minute after RI injection of the *E. coli* preparation, the bile contained high radioactive counts in normal rats, showing mean values of 25 669 cpm (ranges 34 373 - 179 942 cpm). Whereas three of 4 obstructed rats showed lower counts in bile, thus mean values in obstructed rats were 11 198 cpm (ranges 36 988 - 2 451 cpm). The decline of radioactivity from 1 min to 2 min was steep in normal rats. The radioactivity after 2 min declined exponentially for the following 10 min in both normal and obstructed rats (Fig. 1). Bile samples collected from 2 min to 15 min af-

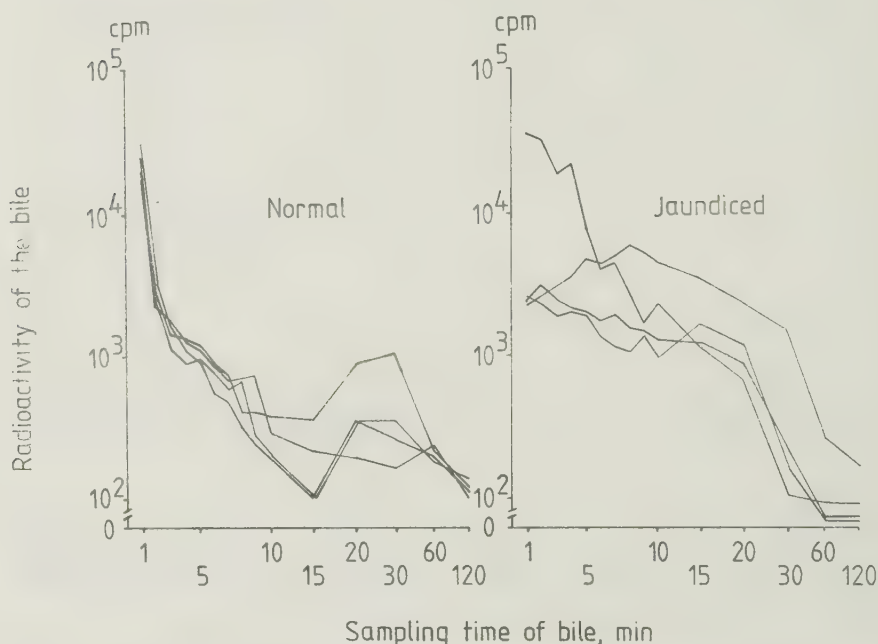


Fig. 1. Clearance of ^{125}I -labelled heat-killed *E. coli* from the bile in 4 normal rats (left) and 4 rats with 3 weeks' obstruction of the common bile duct (right). The *E. coli* preparation, 0.1 ml, containing 2×10^5 counts per minute (cpm) was injected in the biliary tree and samples of 10 μl were collected at times indicated on the abscissa. Ordinate: radioactivity in cpm in the bile samples (log scale).

ter injection showed in all 4 rats with biliary obstruction higher amounts of radioactivity than in the 4 normal animals ($p < 0.05$). At 15 min, for example, the normal rats exhibited a mean value of 236 cpm/10 μl sample, to be compared to a mean of 2 015 cpm in the animals with bile obstruction. When the radioactivity excreted during the first 15 min by the two groups of animals was estimated by the calculation of the area "below the curves" in Fig. 1, the obstructed rats excreted more *E. coli*

than the normal rats. However, the clearance rates were significantly higher in the normal rats; mean $K = 0.1158$ (ranges $0.1394 - 0.0869$), to be compared to 0.0473 (ranges $0.0793 - 0.0061$) in the obstructed rats ($p < 0.05$).

After 15 min, no significant differences were recorded between the obstructed and the normal rats with respect to the radioactivity in the bile. Notably, three of 4 normal rats secreted transiently more radioactivity at 20 - 30 min than at 15 min after RI injection of the bacteria (Fig. 1).

Blood samples were taken simultaneously together with the collection of bile specimens. None of them exhibited a radioactivity exceeding the background count.

DISCUSSION

The data presented here concerned the first study of the kinetics of bacterial clearance from the bile tract. A clear-cut difference was found between normal rats and rats with chronic biliary obstruction. Thus, rats with chronic obstruction showed a markedly decreased clearance rate of E. coli as compared to normal rats. This difference could not be ascribed to a more rapid loss of radioactive E. coli via the CBD in normal rats, since the obstructed rats, taking also into account of the increased bile flow, excreted far more radioactivity than normal animals during the first 15 min after injection of bacteria. It can therefore be concluded that the bacteria were rapidly cleared off the bile in the normal rats due to attachment to, or phagocytosis by, some of the cells surrounding the hepatobiliary tree. At 15 min after RI injection, the obstructed rats harboured almost 10 times as much bacteria in the bile as the normal group. This impaired clearance function can obviously be crucial for the establishment of cholangitis in chronic biliary obstruction, once living bacteria are introduced in the bile.

The absence of radioactivity in the blood from the normal as well as obstructed rats following RI injection of ^{125}I -labelled E. coli during the experimental period of 2 hours suggested that the initial regurgitation of bacteria to the blood stream has little significance to the establishment of biliary sepsis in experimental models involving RI injection of living E. coli.

Jacobsson et al. (7) found in one experiment in a dog that most of the RI-injected radioactive bacteria were filtered off in the liver, but suggested that 10 per cent might have entered the blood stream. The latter possibility, however, could not be further elucidated due to the low radioactivity in the blood in the present study.

The initial radioactivity of the bile (10 μl) was lower in obstructed rats than in normal except in one case. The difference was considered due to the dilution of radioactive substance in the dilated CBD. The second peak of radioactivity occurring 20-30 min after injection of the bacteria in three of 4 normal rats (Fig. 1) might reflect an excretion of bacterial products, or metabolites from the break-down of the microorganisms.

Peterson and Fujimoto (10) who injected ^{14}C -morphine in the CBD of rats showed that a radioactive derivative, morphine-3-glucuronide, appeared in the bile in a second peak following ^{14}C -morphine. Further studies will be needed to elucidate the processes leading to the delayed increase in radioactivity in the normal rats, before we could ascribe the phenomenon to the bacterial metabolism.

In conclusion, rats with chronic biliary obstruction showed a markedly impaired capacity to clear bacteria from the bile. This finding might have relevance to the development of biliary sepsis. The present model might be useful for studies of the pathological processes leading to increased susceptibility to bacterial infections in patients with obstructive jaundice.

REFERENCES

1. Barber-Riley, G.: Rat bile tree during reflux perfusion. *Am. J. Physiol.* 210: 1137-1141 (1965).
2. Drivas, G.; James, O.; Wardle, N.: Study of reticuloendothelial phagocytic capacity in patients with cholestasis. *Br. Med. J.* 1: 1568-1569 (1976).
3. Freedman, L.R.: The relationship between bacterial infection of the hepato-biliary system and common bile duct obstruction in man. *Yale J. Biol. Med.* 35: 318-327 (1963).
4. Ho, K.; Drummond, J.L.: Circadian rhythm of biliary excretion and its control mechanisms in rats with chronic biliary drainage. *Am. J. Physiol.* 229: 1427-1437 (1975).
5. Holman, J.M.; Rikkers, L.F.: Biliary obstruction and host defense failure. *J. Surg. Res.* 32: 208-213 (1982).
6. Jackaman, F.R.; Hilson, G.R.F.; Lord Smith of Marlow: Bile bacteria in patients with benign bile duct stricture. *Br. J. Surg.* 67: 329-332 (1980).
7. Jacobsson, B.; Kjellander, J.; Rosengren, B.: Cholangiovenous reflux. An experimental study. *Acta Chir. Scand.* 123: 316-321 (1962).
8. Katz, S.; Grosfeld, J.L.; Gross, K.; Plager, D.A.; Ross, D.; Rosenthal, R.S.; Hull, M.; Weber, T.R.: Impaired bacterial clearance and trapping in obstructive jaundice. *Ann. Surg.* 199: 14-20 (1984).
9. McConahey, P.J.; Dixon, F.J.: A method of trace iodination of proteins for immunologic studies. *Int. Arch. Allergy* 29: 185-189 (1966).
10. Peterson, R.E.; Fujimoto, J.M.: Retrograde intrabiliary injection: Absorption of water and other compounds from the rat biliary tree. *J. Pharmacol. Exp. Ther.* 185: 150-162 (1973).
11. Scott, A.J.; Khan, G.A.: Origin of bacteria in the bile duct bile. *Lancet* ii: 790-792 (1967).
12. Tanaka, N.; Christensen, P.; Rydén, S.; Klöfver-Ståhl, B.; Bengmark, S.: A rat model for sepsis in chronic biliary obstruction. Submitted for publication.
13. Trams, E.G.; Symeonidis, A.: Morphologic and functional changes in the livers of rats after ligation or excision of the common bile duct. *Am. J. Path.* 33: 13-27 (1957).
14. Vonk, R.J.; Van Doorn, A.B.D.; Strubbe, J.H.: Bile secretion and bile composition in the freely moving, unanaesthetized rat with a permanent biliary drainage: influence of food intake on bile flow. *Clin. Sci. Mol. Med.* 55: 253-259 (1978).

Postal address: N. Tanaka, M.D.
Department of Surgery
University of Lund
S-221 85 LUND
Sweden

IMPAIRED LIVER CLEARANCE OF BACTERIA IN RATS WITH CHRONIC BILIARY
OBSTRUCTION

N Tanaka¹, P Christensen², S Rydén¹, B Klöfver-Ståhl¹ and S Bengmark¹

Department of Surgery¹ and Department of Medical Microbiology²,
University of Lund, S-221 85 LUND, Sweden

Running title: Bacterial clearance from the bile in biliary obstruction

Summary

^{125}I -labeled E. coli was injected into the biliary tree of normal rats and rats with 3 weeks' obstruction of the common bile duct, to investigate the liver clearance capacity for bacteria. Bile was collected during 15 min, immediately, 1 h, 4 h or 24 h after the injection. Tissue specimens from the liver, lungs, spleen and kidneys, and blood and urine specimens were collected simultaneously. In normal rats, 40 % of the bacteria was recovered in the bile immediately after the injection, whereas 30 % already was trapped in the liver. Incubation of the bacteria in the bile duct for 1 h, 4 h and 24 h resulted in liver retentions of 43 %, 15 % and 4 %, respectively. The recovery in the bile was 13 % after 1 h incubation and further prolongation of the incubation did not result in a significant decrease. In contrast to these findings 70 % of the injected bacteria was retained in the biliary tree in rats with chronic biliary obstruction ($p < 0.05$ compared to normal rats) and only 1 % was trapped in the liver ($p < 0.005$) 15 min after injection. One hour incubation of bacteria in the bile duct decreased the retention in the bile to 30 % but the retention in the liver increased only slightly in these animals. Four and 24 h after injection less than 30 % of the bacteria was retained in the hepato-biliary system. Most of these animals showed almost no radioactivity exceeding the background count in the blood, urine, spleen, lungs and kidneys 15 min after injection. It was concluded that the impaired clearance capacity of the liver in chronic biliary obstruction might contribute to the susceptibility of such animals for bacteria introduced in the bile.

Key words: Biliary sepsis - ^{125}I -labeled E. coli - Retrograde intra-biliary injection - Biliary obstruction - Rat

INTRODUCTION

Obstructive jaundice predisposes to biliary sepsis (3, 8, 13). These biliary infections are serious threats to the patients' lives. The mortality rate in secondary septicemia has been reported to range between 9 and 40 % (15). In spite of extensive research, the mechanisms leading to septicemia in obstructive jaundice are still not fully understood.

According to one theory blood-borne bacteria reach the bile by penetrating through minor tissue injuries in the bile ducts (1, 12). Ascending colonization with bacteria from the duodenum might be another route of acquisition (16). Whatever the route of acquisition of bacteria it may be, man as well as animals with chronic biliary obstruction are highly susceptible to biliary infection. Thus, in a recent study by us the majority of rat with chronic biliary obstruction died upon retrograde intrabiliary (RI) injection of 10^5 E. coli whereas normal rats resisted a RI challenge of 10^8 E. coli although the common bile duct (CBD) was ligated in connection with the injection (14a). However, the background to the immunodeficiency in rats with biliary obstruction remains to be elucidated.

The regurgitation of bile constituents into the blood in biliary obstruction has been attributed to either transcellular, paracellular, or necrotic hepatic pathways (2, 4, 7, 11). Although passages of large particles as bacteria must be more difficult than bile constituents, a similar spreading mechanism for bacteria can not be excluded a priori. Mixer et al (10) and Hultborn et al (5) demonstrated a cholangiovenous reflux of bacteria during cholangiography. Choledochal contents were suggested to regurgitate at pressure insignificantly higher than the secretion pressure (5).

Another factor of crucial importance in the prevention of establishment and growth of bacteria in the biliary tree is the capacity for clearance of bacteria from the biliary tract. We have recently studied the clearance of ^{125}I -labeled E. coli from the rat bile and showed a characteristic decreased capacity for bacterial clearance in animals with chronic CBD obstruction (14b). The aim of the present study was to investigate the mechanism behind the impaired clearance capacity in the chronically jaundiced animals. The possibility of regurgitation of bacteria from the bile to the blood was also studied.

MATERIALS AND METHODS

Animals. Fifty-two male Sprague-Dawley rats initially weighing 250-300 g were used. The animals were maintained on an artificial day and night cycle and fed laboratory food (pellets) and water ad libitum.

Obstruction of the CBD. Extrahepatic obstruction of the CBD was created by double ligation and excision of the CBD between the lowermost tributary of the bile duct and the uppermost tributary of the pancreatic duct in 20 animals 3 weeks before RI injection of E. coli (see below). Thirty-two animals without ligation of the CBD served as controls.

Radiolabeling of bacteria. A preparation of a heat-killed *E. coli* strain was radiolabeled with ^{125}I according to the protein-labeling method of McConahey and Dixon (9) as previously described (14b). The radioactivity was approximately 2.0×10^6 counts per minute (cpm)/ml bacterial suspension.

RI injection of radiolabeled *E. coli*. Each experiment was performed in the morning under light ether anesthesia using clean but not sterile conditions. The rectal temperature of the animals was maintained between 37°C and 37.5°C .

After laparotomy, the CBD was cannulated with a 20 cm silastic catheter (Dow Corning Corporation, Michigan, USA; volume approximately 18 μl , the tip of which was placed below the lowermost tributary of the bile duct in order not to interfere with the flow to the papillary processes. In the obstructed rats, the catheter was secured with a purse-string suture in the cystically dilated CBD, making efforts to minimize the leakage of bile and thereby the decrease in biliary pressure. Injection of bacteria was performed via the bile duct cannula using an infusion pump (Harvard Apparatus Company, Milis, Mass, USA; Model 840), which delivered 118 μl solution (including the volume of the cannula) to each rat with a rate of 32.8 $\mu\text{l}/\text{min}$. This rate was chosen since preliminary experiments showed that a more rapid infusion increased the biliary pressure more than twice above the resting pressure in normal rats. Volumes above 100 μl (net) were avoided as the distended capacity of the normal rat biliary tree in a 10 g liver has been shown to be about 120 μl (18).

Following injection of the bacterial suspension, the bile duct cannula was immediately detached from the infusion apparatus. In 8 normal rats and in 5 rats with chronic CBD obstruction, collection of the bile was commenced immediately after injection. In the remaining rats, the cannula was immediately occluded at their distal end deposited under the skin of the abdominal wall for 1, 4 or 24 h until the time of measurement (8 normal and 5 rats with chronic CBD obstruction in each of the three groups).

Measurement of bacterial clearance and organ distribution. Previous experiments have shown that some normal rats exhibit a transient peak of radioactivity in the bile at about 20 min after injection of ^{125}I -labeled *E. coli*, presumably due to the excretion of bacterial metabolites (14b). For bile recovery experiments, the time for collection of the bile specimens was therefore limited to 15 min in the control rats. In the rats with chronic biliary obstruction, collection of the bile was also performed for 15 min, but this volume was pooled together with bile subsequently aspirated from the distended bile duct. Following collection of bile, the rats were immediately exsanguinated by aortic puncture. After excision of the extrahepatic bile duct, the liver was removed and weighed, as were the spleen, lungs and kidneys. Blood (0.5 ml) and urine (0.5 ml) were also collected.

The radioactivity recovered in the bile and the extrahepatic bile duct was summed and expressed in per cent of the total amount of ^{125}I -labeled *E. coli* injected to the rat. Tissue samples (200-500 mg) were cut out of

the organs for measurement of radioactivity, and the final distribution of bacteria in the different organs was expressed in per cent of the injected bacteria, per gram of tissue or in the total organ. Preliminary experiments demonstrated an uneven distribution of the radiolabeled *E. coli* between the different liver lobes (Fig 1). At least three slices of tissue from each lobe were therefore collected and each lobe weighed separately to obtain a representative picture of the bacterial uptake.

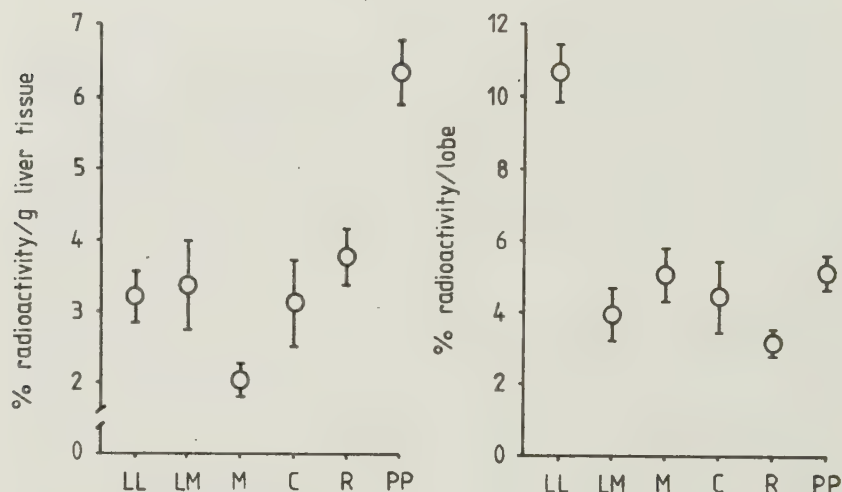


Fig 1. Distribution of radioactivity in 6 different liver lobes after retrograde intrabiliary injection of ^{125}I -labeled *E. coli*. Ordinate: results expressed in per cent of the injected dose of bacteria, per gram of liver tissue (left) or per liver lobe (right). The mean values obtained for 5 normal rats are shown. Bars indicate SEM. LL: left lateral; LM: left medial; M: medial; C: caudate; R: right; PP: papillary processes.

The radioactivity of the samples was determined using a 1260 Multigamma counter (LKB, Stockholm, Sweden), with a background not exceeding 60 cpm.

Statistical method. The results were expressed as the mean $\pm$ SEM. The data were evaluated using Student's *t* test unless otherwise indicated.

RESULTS

In normal rats, 40 % of the bacteria was recovered in the bile during the 15 min collection period following the injection of bacteria, whereas 30 % was already trapped in the liver. Occlusion of the CBD for 1 h after RI injection of bacteria in normal rats reduced the recovery in the bile to 13 % and increased the retention in the liver to 43 %. Fur-

ther prolongation of the occlusion resulted in a slight decrease of the radioactivity in the bile, but the retention in the liver decreased gradually to 15 % after 4 h and to 4 % after 24 h occlusion (Fig 2).

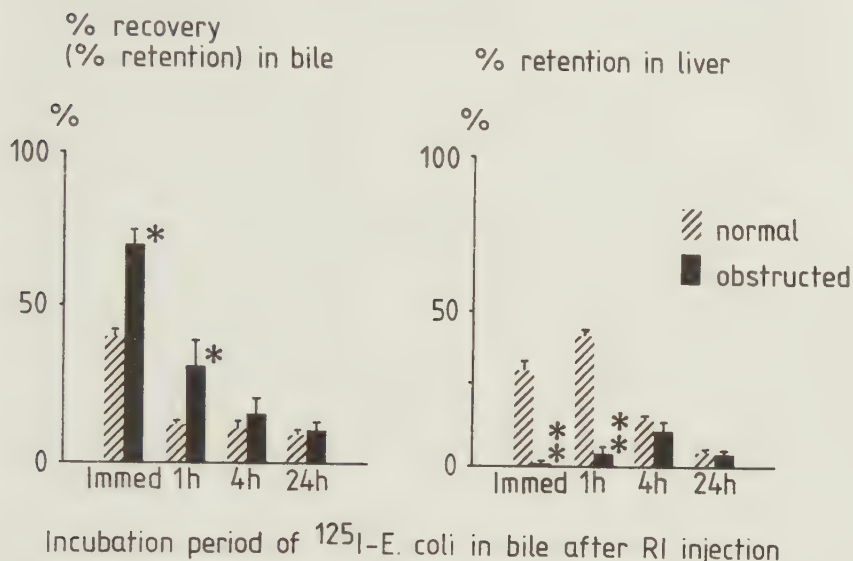


Fig 2. Recovery of the radioactivity in the bile/biliary tree (left) and retention in the liver (right) in normal rats (hatched bar) and rats with chronic biliary obstruction (closed bar) after retrograde intrabiliary injection of ^{125}I -labeled *E. coli*. Abscissa: incubation period (Immed: immediately after injection, 1 h, 4 h and 24 h) after retrograde intrabiliary injection. Ordinate: radioactivity in per cent of the injected dose of bacteria. Each mean value was obtained from 8 normal rats and 5 obstructed rats. Bars indicate SEM. * $p < 0.05$ compared to normal rats. ** $p < 0.005$ compared to normal rats.

The initial trapping of bacteria in the liver in the rats with chronic biliary obstruction was significantly impaired compared to normal rats. More than 70 % of the injected bacteria was retained in the biliary tree 15 min after injection ($p < 0.05$ compared to normal rats) and only 1 % was trapped in the liver ($p < 0.005$ compared to normal rats). One hour incubation of bacteria in the CBD increased the retention of bacteria in the liver, but it was still significantly less than in normal rats. Four and 24 h after RI injection of bacteria less than 30 % was retained in the hepato-biliary system in both groups of animals (Fig 2).

Most of the animals showed almost no radioactivity exceeding the background count in the blood, urine, spleen, lungs and kidneys 15 min after injection of bacteria. Although the radioactivity was low, five of the 8 normal animals, however, showed significant radioactivity in the lungs

(207-1378 cpm in the total organ) 1 h after RI injection of bacteria compared to the chronically obstructed rats, in which none showed radioactivity in the lungs ($p = 0.044$, Fisher's exact probability test).

Prolongation of the incubation period in the CBD in chronically obstructed rats did not result in a detectable increase in the radioactivity in the spleen, lungs or kidneys (the radioactivity in these organs was less than 0.4 % of the injected dose).

DISCUSSION

The data demonstrated that only 40 % of the radioactive E. coli deposited into the CBD of normal rats could be recovered in the biliary tree 15 min after injection, whereas 30 % was trapped in the liver. The localization of bacteria within the microstructure of the liver was not further studied. However, the finding that only 1 % of the bacteria could be recovered in the liver of the chronically CBD-obstructed animals immediately after the RI injection suggested that the background of radioactivity due to contamination with bacteria present in the bile was very low. The process of bacterial uptake in the liver of the normal rats continued for at least 1 h. The following decrease in radioactivity 4-24 h after injection indicated that bacterial products were released from the liver. Since the radioactivity in the bile of the normal rats was not changed substantially between 1 and 24 h incubation, few breakdown substances appeared to be secreted in the bile during this period.

The clearance of bacteria from the bile in the chronically jaundiced rats exhibited a striking contrast to the normal rats, as previously described (14b). The radioactivity recovered in the bile and the extrahepatic biliary tree after RI injection of radiolabeled E. coli in chronic obstruction indicated that more than 70 % of the injected dose was retained in the biliary tree. Thus, the capacity of the liver to take up biliary bacteria was greatly impaired. Incubation of bacteria in the CBD for several hours enhanced the uptake only a little. Nevertheless the retention of bacteria in the bile decreased with prolonged incubation, suggesting that the liver was quite leaky for bacteria present in the biliary tree. This situation might be crucial for the development of biliary sepsis in rats with chronic CBD obstruction.

The present study was not designed to elucidate the fate of the bacteria after leaving the liver. The radioactivity of the RI injected bacteria was too low to show the meaningful localization of bacteria in several organs since the distribution of bacteria in the rat body diluted the radioactivity to counts close to the background in most cases. Although it could be demonstrated that the uptake of the radioactivity in the lungs was higher than in normal rats 1 h after RI injection than in chronically obstructed rats, it might reflect the uptake of the bacterial breakdown products by the lungs in normal rats. The significance of the finding awaits further investigation.

It has been recognized for many years that acute ligation of the CBD and/or complete occlusion due to malignancy causes fewer incidences of biliary sepsis than benign or partial obstruction (3, 6). The reason

has not been fully determined. The present results might provide a plausible explanation. Because of the rapid process in acute, complete obstruction, the liver reticuloendothelial system can be regarded as almost intact at the time of bile duct occlusion. Thus, the bacteria present in the occluded bile duct can effectively and immediately be taken up by the liver.

We conclude from the present study that the rats with chronic CBD obstruction have an impaired capacity of the liver to clear the bacteria from the biliary tree. This may contribute to the susceptibility of such animals to bacteria introduced in the bile.

REFERENCES

1. Chou ST, Gibson JB (1968) Experimental cholangitis and cholelithiasis. *Brit J Exp Path* 49: 565-573
2. Devos R, Desmet VJ (1978) Morphologic changes of the junctional complex of the hepatocytes in rat liver after bile duct ligation. *Brit J Exp Path* 59: 220-227
3. Flemma RJ, Flint LM, Osterhout S, Shingleton WW (1967) Bacteriologic studies of biliary tract infection. *Ann Surg* 166: 563-572
4. Hampton JC (1961) Electron microscopic study of extrahepatic biliary obstruction in the mouse. *Lab Invest* 10: 502-513
5. Hultborn A, Jacobsson B, Rosengren B (1962) Cholangiovenous reflux during cholangiography. An experimental and clinical study. *Acta Chir Scand* 123: 111-124
6. Jackaman FR, Hilson GRF, Lord Smith of Marlow (1980) Bile bacteria in patients with benign bile duct stricture. *Br J Surg* 67: 329-332
7. Layden TJ, Elias E, Goyer JL (1978) Bile formation in the rat: The role of the paracellular shunt pathway. *J Clin Invest* 62: 1375-1385
8. Maddocks AC, Hilson GRF, Taylor R (1973) The bacteriology of the obstructed biliary tree. *Ann Royal Coll Surgeons* 52: 316-319
9. McConahey PJ, Dixon FJ (1966) A method of trace iodination of proteins for immunologic studies. *Int Arch Allergy* 29: 185-189
10. Mixer HW, Rigler LG, Oddone MVG (1947) Experimental studies on biliary regurgitation during cholangiography. *Gastroenterology* 9: 64-80
11. Rolland A, Bioulac P, Saric J, Balabaund C (1982) Early patchy hepatic necrosis; its appearance after bile duct ligation in rats with a portocaval shunt. *Arch Path Lab Med* 106: 150-152
12. Ronai PM, Magarey FR (1960) Experimental cholangitis following biliary obstruction. *Australasian Ann Med* 9: 289-294
13. Scott AJ, Khan GA (1967) Origin of bacteria in bile duct bile. *Lancet* ii: 790-792
- 14a. Tanaka N, Christensen P, Rydén S, Klöfver-Ståhl B, Bengmark S (accepted for publication) A rat model for sepsis in chronic biliary obstruction. *Acta microbiol et path scand*
- 14b. Tanaka N, Christensen P, Rydén S, Klöfver-Ståhl B, Bengmark S (accepted for publication) Decreased clearance of Escherichia coli from the bile in rats with obstructive jaundice. *Eur Surg Res*

15. Thompson JE, Thompkins RK, Longmire WP (1982) Factors in management of acute cholangitis. Ann Surg 195: 137-145
16. Williams R, Dawson JL (1969) The management of ascending cholangitis. Proc Royal Soc Med 62: 243-245

PROTECTIVE ROLE OF THE SPLEEN AGAINST BILIARY SEPSIS IN THE RAT

Nobutaka Tanaka¹, Poul Christensen², Stefan Rydén¹, Britten Klöfver-Ståhl¹ and Stig Bengmark¹

¹Department of Surgery and ²Department of Medical Microbiology,
University of Lund, S-221 85 LUND, Sweden

Abstract

The effect of splenectomy on the susceptibility of rats to retrograde intrabiliary (RI) or intravenous (IV) injection of 10^5 *Escherichia coli* was studied. IV injection of bacteria in otherwise normal, splenectomized animals did not cause any deaths, independent on whether simultaneous ligation of the common bile duct was performed or not. In contrast, RI injection immediately after splenectomy resulted in death in 9/12 animals although only 1/12 RI injected rats with intact spleens died ($p < 0.005$). The mortality rates after RI injection of *E. coli* in splenectomized (otherwise normal) rats and rats with 3 weeks' biliary obstruction did not differ; however, the median survival time in the former group was significantly longer (5 days) than in the latter (18 h) ($p < 0.05$). The results indicated that the spleen plays a crucial role for protection against biliary sepsis. The findings might have implications for the handling of patients with combined hepato-biliary and splenic diseases.

Key words: Biliary sepsis; *Escherichia coli*; splenectomy; retrograde intrabiliary injection; biliary obstruction; rat.

Nobutaka Tanaka, Department of Surgery, University of Lund, S-221 85 LUND, Sweden.

INTRODUCTION

The clearance mechanism for bacteria in the hepato-biliary system is greatly hampered in rats with chronic biliary obstruction (15). Although such an impaired capacity might explain the proneness to biliary sepsis in chronic biliary obstruction (16), the relative contributions of other reticuloendothelial system (RES) organs to protection against biliary sepsis remain to be determined.

The spleen plays a role for clearance of blood-borne particulate antigens, in cell-mediated immunity and for production of antibody and other opsonins (5, 7, 11, 12). In the absence of specific antibody, the spleen constitutes the most effective clearance organ for bacteria (2). Splenectomy therefore results in increased susceptibility to bacterial infection.

Following retrograde intrabiliary (RI) injection of bacteria, increased mortality was observed in rats with chronic biliary obstruction, whereas normal rats survived (16). Accordingly, we considered it interesting to investigate whether splenectomy affects the resistance to RI injection of bacteria in normal rats. Such studies might have practical consequences for the handling of splenectomy in patients who are predisposed to develop biliary sepsis. Furthermore, the results could point to possible risks for biliary infections in splenectomized individuals.

MATERIALS AND METHODS

Animals

Sixty-six male Sprague-Dawley rats initially weighing 250-270 g were used.

Experimental design

The animals were divided into the following 6 groups according to the operative procedures and the route of bacterial challenge (see below): Twelve rats were splenectomized (Group I) and 12 were sham-operated (Group II) and both received RI injection of bacteria in connection with ligation of the common bile duct (CBD) immediately after the operation. Another 12 rats (Group III) underwent a sequence of splenectomy, CBD ligation and intravenous (IV) injection of bacteria. Twelve splenectomized (Group IV) and 12 sham-operated rats (Group V) were given bacteria intravenously immediately after the operation without ligation of the CBD. Six rats with 3 weeks' biliary obstruction (Group VI) received RI injection of bacteria without performing splenectomy (Table I).

Surgical procedures

All operations including injections of bacteria (see below) were performed under ether anesthesia using clean but not sterile conditions. Splenectomy was performed via a midline laparotomy after mobilization of the spleen and individual ligation of the splenic vessels. The sham-operation included splenic mobilization and resection of the greater omentum

Table I. Outcome of the challenge with 10^5 colony forming units (CFU) *E. coli*

Group	CBD obstruction ^a	Splenectomy	Route of challenge ^b	No. of killed rats/ no. of challenged rats
I	+	+	RI	9/12 ^c
II	+	-	RI	1/12
III	+	+	IV	0/12
IV	-	+	IV	0/12
V	-	-	IV	0/12
VI	+	-	RI	6/6 ^d

^a CBD (common bile duct) obstruction in groups I, II and III was performed in connection with bacterial challenge, whereas the CBD was obstructed 3 weeks before challenge in Group VI

^b RI: retrograde intrabiliary injection. IV: intravenous injection

^c $p < 0.005$ compared to groups II-V; not significantly different from Group VI

^d $p < 0.001$ compared to groups II-V

after individual ligation of the omental vessels. Extrahepatic biliary obstruction for production of chronic jaundice was created by double ligation of the CBD and excision of 5-8 mm of the middle part of the duct between the ligatures to prevent recanalization.

Bacterial challenge

For bacterial challenge, an *E. coli* strain was frozen in aliquots at -80°C (16). The bacteria were thawed immediately before use and diluted in Todd Hewitt broth to obtain 0.1 ml volumes containing 10^5 colony forming units (CFU).

RI injection of bacteria was performed as previously described (16). In brief, in normal rats (Group I and II), 0.1 ml bacteria was slowly injected proximally to a ligature applicated on the CBD just above the entrance of the highest branch of the pancreatic duct. After injection, the CBD was again ligated proximally to the needle hole with another thread to prevent leakage of bacteria. In the rats with chronic CBD obstruction (Group VI), 0.1 ml bile was withdrawn for culture. A purse-string suture applicated on the dilated bile duct prevented leakage of bacteria after injection in these animals. For IV injection of bacteria, the lateral vein of a hind limb or the penile vein was used.

After challenge, the animals were kept under standard laboratory condi-

tions with food (pellets) and water *ad libitum*, and observed every 6th h for 7 days. Surviving rats were sacrificed 7 days after the challenge. Succumbed and surviving rats were autopsied and cultures from heart blood and bile of all animals were collected and processed on hematin agar plates as previously described (16).

Statistical method

Survival times for the various groups of killed animals were compared using *Mann-Whitney's* rank sum test for unpaired data. The mortality rates were evaluated using *Fischer's* exact (probability) test.

RESULTS

RI injection of bacteria into rats with chronic biliary obstruction (Group VI) caused a 100 % mortality within 24 h. This was significantly different from the results with the normal rats (Group II) in which RI injection of bacteria was performed immediately after ligation of the CBD ($p < 0.001$; Table I). Bile cultures from the rats in Group VI taken immediately before the bacterial challenge were all negative.

The mortality increased to 75 % when splenectomy was performed immediately before RI injection in normal rats (Group I; $p < 0.005$ compared to Group II). This rate did not differ statistically from that in the chronically jaundiced animals (Group VI); however, the median survival time among killed animals in Group I was significantly longer (125 h; range 18-158 h) than in Group VI (18 h; range 16-21 h) ($p < 0.05$).

IV injection of the same dose of bacteria in splenectomized as well as sham-operated rats with or without CBD obstruction (Group III, IV, V) did not cause any signs of sepsis and none of the animals died during the post-operative observation period (Table I).

Autopsy of the succumbed animals invariably revealed congested and hemorrhagic lungs. Blood and bile cultures from all the succumbed rats were positive for *E. coli*. Among the surviving rats, the 8 RI injected rats (Group I, II) showed positive bile cultures.

DISCUSSION

The data demonstrated that the spleen plays a crucial role for protection against biliary sepsis in the present rat model. Thus, RI injection of 10^5 *E. coli* resulted in a high mortality in the splenectomized rats. In contrast, RI injection of the same dose of bacteria in sham-operated animals did not cause sepsis, in accordance with a previous study (16). The combined effect of RI injection and splenectomy was illustrated by the fact that all rats undergoing combined splenectomy and CBD obstruction survived IV challenge with 10^5 *E. coli*.

The increased susceptibility to RI injected bacteria in rats with 3 weeks' CBD obstruction could probably be explained by a decreased clearance rate for bacteria in the hepato-biliary system (15). Although the mortality rates did not differ, the splenectomized rats survived sig-

nificantly longer time after RI injection than the chronically obstructed rats. The delayed course of infection suggested that the bacteria had to grow and multiply in the bile during a few days to establish a biliary sepsis. This view is in accordance with a previous study in which rats with 3 days' biliary obstruction showed a higher susceptibility to RI injection of bacteria than normal rats immediately after ligation of the CBD (17). The combination of this process with the impaired blood clearance capacity for particulate antigens would be a plausible mechanism for the susceptibility of splenectomized rats to RI injection of *E. coli*. Autopsy revealed that the lungs of the succumbed, splenectomized rats invariably showed congested, hemorrhagic appearance, suggesting an increased entrapment of the bacteria in the lungs (3, 4). The lungs could probably not deal with the overload of bacteria (10) in the absence of the spleen in the biliary obstructed animals.

Overwhelming postsplenectomy infections in humans as well as animals are known to be caused mainly by encapsulated bacteria (1, 6, 14). *Streptococcus pneumoniae* is responsible for about 50 % of the septic events in splenectomized individuals. *E. coli* is reported to be responsible for 12 % of the postsplenectomy sepsis cases (14). Chaudry et al (4) and Scher et al (13) in their recent works emphasized the impaired capacity for clearance of such bacteria after splenectomy. Previous studies from our laboratory, using *Streptococcus pneumoniae* type 1, demonstrated that rats could develop overwhelming infection immediately after splenectomy (8). The fact that IV injection of *E. coli* did not kill any of the animals in the present study might therefore be explained by the relatively low dose of injected bacteria.

Although available data indicated that splenectomy has no significant influence on mortality rate in the rats with established biliary obstruction (9), the present study demonstrated the protective role of the spleen in biliary sepsis. This finding points to the importance of antibiotic prophylaxis in patients who undergo splenectomy and are prone to develop biliary infections.

REFERENCES

1. Alumark, A., Bengmark, S., Gullstrand, P. & Schalén, C.: Improvement of the splenectomized rat model for overwhelming pneumococcal infection. Standardization of the bacterial inocula. Eur. Surg. Res. 13: 339-343, 1981
2. Benacerraf, B., Sebestyen, M.M. & Schlossman, S.: A quantitative study of the kinetics of blood clearance of P32-labelled *Escherichia coli* and *Staphylococci* by the reticuloendothelial system. J. Exp. Med. 110: 27-48, 1959
3. Bogart, D., Biggar, W.D. & Good, R.A.: Impaired intravascular clearance of *Pneumococcus type 3* following splenectomy. J. Res. 11: 77-87, 1972
4. Chaudry, I.H., Tabata, Y., Schleck, S. & Baue, A.E.: Effect of splenectomy on reticuloendothelial function and survival following sepsis. J. Trauma 20: 649-656, 1980
5. Claret, I., Morales, L. & Montaner, A.: Immunological studies in the postsplenectomy syndrome. J. Ped. Surg. 10: 59-64, 1975
6. Cooney, D.R., Dearth, J.C., Swanson, S.E., Dewanjee, M.K. & Telander, R.L.: Relative merits of partial splenectomy, splenic reimplantation, and immunization in preventing postsplenectomy infection. Surgery 86: 561-569, 1979
7. Ellis, E.F. & Smith, R.T.: The role of the spleen in immunity with special reference to the postsplenectomy problem in infants. Pediatrics 37: 111-119, 1966
8. Gullstrand, P., Alumark, A., Schalén, C. & Christensen, P.: Overwhelming pneumococcal infection in rats immediately after splenectomy. J. Surg. Res. 33: 220-224, 1982
9. Holman, J.M. & Rikkers, L.F.: Biliary obstruction and host defense failure. J. Surg. Res. 32: 208-213, 1982
10. Katz, S., Grosfeld, J.L., Gross, K., Plager, D.A., Ross, D., Rosenthal, R.S., Hull, M. & Weber, T.R.: Impaired bacterial clearance and trapping in obstructive jaundice. Ann. Surg. 199: 14-20, 1984
11. Likhite, V.V.: Immunological impairment and susceptibility to infection after splenectomy. JAMA 236: 1376, 1976
12. Rowley, D.A.: The formation of circulating antibody in the splenectomized human being following intravenous injection of heterologous erythrocytes. J. Immunol. 65: 515-521, 1950
13. Scher, K.S., Wroczynski, F. & Coil, J.A.: The effect of splenectomy on gram-negative bacteremia. J. Trauma 22: 407-409, 1982

14. *Singer, D.B.*: Postsplenectomy sepsis. *Perspect. Pediat. Path.* 1: 285-311, 1973
15. *Tanaka, N., Christensen, P., Rydén, S., Klöfver-Ståhl, B. & Bengmark, S.*: Impaired liver clearance of bacteria in rats with chronic biliary obstruction. *Res. Exp. Med.* submitted 1984
16. *Tanaka, N., Christensen, P., Rydén, S., Klöfver-Ståhl, B. & Bengmark, S.*: A rat model for sepsis in chronic biliary obstruction. *Acta Path. Microbiol.* in press
17. *Tanaka, N., Christensen, P., Rydén, S., Klöfver-Ståhl, B. & Bengmark, S.*: Biliary obstruction and susceptibility to biliary sepsis in rats. *Res. Exp. Med.* in press

ROLE OF CIRCULATING ANTIBODIES IN BACTERIAL CLEARANCE IN RATS WITH
CHRONIC BILIARY OBSTRUCTION

Nobutaka Tanaka, Poul Christensen, Stefan Rydén, Britten Klöfver-Ståhl
and Stig Bengmark

*From the Department of Surgery and Department of Medical Microbiology,
University of Lund, Sweden*

Correspondence to Dr. Nobutaka Tanaka, Kirurgiska kliniken, Lasarettet,
S-221 85 LUND, Sweden

Abstract. Biliary obstruction was created in male Sprague-Dawley rats and a suspension of heat-killed *E. coli* strain was injected intravenously at three occasions in the following week. Three weeks after the CBD-obstruction, IgG antibodies to surface antigens of the strain were measured using ^{125}I -labeled protein A. A more than 20-fold increase was noted in the vaccinated rats. However, retrograde intrabiliary (RI) injection of 10^5 colony forming units of the *E. coli* strain resulted in a high mortality in chronic obstructed rats (9/10 and 10/10 died, respectively), irrespective of whether the rats were immunized or not. The blood clearance and organ localization of the *E. coli* was determined by intravenous injection of ^{125}I -labeled *E. coli*. The lung uptake of bacteria was significantly increased in non-immunized obstructed rats as compared to sham operated rats ($p < 0.005$). Immunization of the rats with CBD obstruction increased the lung uptake further ($p < 0.005$) compared to non-immunized obstructed rats. The results demonstrated that circulatory specific antibodies in CBD obstructed rats increased the uptake of bacteria in the lungs which might lead to lung failure, thereby explaining the lack of protective effect of vaccination.

Biliary infection is a serious threat to patients with obstructive jaundice. Thus, mortality and morbidity in surgery for biliary diseases associated with jaundice and infected bile are impressively high (12, 14, 25). In order to decrease the operative risk, preoperative percutaneous transhepatic drainage (PTD) as well as prophylactic antibiotic treatment has been advocated (17, 25).

Initially, PTD exhibited a dramatic effect in acute suppurative obstructive cholangitis (ASOC) (22, 27). However, recent trials have failed to demonstrate a reduction in mortality and have shown even higher morbidity compared to non-PTD allocated controls (7, 19, 20), despite a significant reduction of serum bilirubin after PTD. It may also be difficult to obtain an adequate drainage of thick pus through a narrow catheter in ASOC, and contamination with environmental bacteria during drainage is frequently seen (3, 19, 24). Antibiotic prophylaxis, on the other hand, holds an important place in the treatment of biliary sepsis, although the proper prescription which avoids unnecessary prophylaxis and achieves satisfactory therapeutic bile level of antibiotics remains to be defined (13, 15).

Patients with obstructive jaundice exhibit defective reticuloendothelial system (RES) function (5) and, consequently, impaired immunity (11, 23). Furthermore, depression of Kupffer cell activity in chronic biliary obstruction in experimental animals (8, 10) has been suggested to alter their immune competence (8). Improvement of immune defense mechanism in hepato-biliary disease seems therefore to be crucial for protection against bacterial infection.

We have previously demonstrated that rats with chronic biliary obstruction are highly susceptible to retrograde intrabiliary (RI) injection of bacteria (28, 29). Taking advantage in this model, the present paper concerns the effect of vaccination against bacteria in chronic biliary obstruction. The influence of the immunization on blood clearance and organ localization of bacteria in the rats was studied.

MATERIALS AND METHODS

Animals. Fifty-four male Sprague-Dawley rats weighing 290-370 g were used. The animals were maintained on an artificial day and night cycle and fed laboratory food (pellets) and water *ad libitum*.

Experimental groups and surgical procedures. All operations including injections of bacteria (see below) were performed under ether anesthesia using clean but not sterile conditions. Extrahepatic obstruction of the common bile duct (CBD) was created by double ligation and excision of the CBD between the lowermost tributary of the bile duct and the uppermost tributary of the pancreatic duct in 32 animals 3 weeks before injection of radiolabeled *E. coli* (see below). Twenty-two animals were sham-operated (isolation of the CBD without ligation).

Immunization. One week after operation, 16 animals with biliary obstruction and 6 sham-operated animals were immunized with heat-killed *E. coli*. The vaccine was prepared from an overnight culture of a pre-

viously described strain (28) in Todd-Hewitt broth at 37 °C. After incubation, the bacteria were sedimented by centrifugation, washed twice in sterile saline, and resuspended in saline followed by heating at 80 °C for 30 min. After control of the sterility, the bacterial concentration was adjusted by spectrophotometer to an optical density of 1.0 at 540 nm, corresponding to approximately 10^{10} *E. coli* per ml. The immunization consisted of intravenous injection of 0.1, 0.2 and 0.3 ml suspension for three consecutive days, respectively.

Bacterial challenge. Thirty animals (10 immunized and 10 non-immunized rats with 3 weeks' biliary obstruction, as well as 10 sham-operated animals) received RI injections of 0.1 ml *E. coli*, corresponding to 10^5 colony forming units (CFU), as previously described (28). Immediately before injection of bacteria, 0.1 ml of bile was withdrawn for culture. After challenge, the animals were observed every 12th hour for 7 days. Surviving rats were then sacrificed. All rats were autopsied and cultures from heart blood and bile were collected and processed as previously described (28).

Radiolabeling of bacteria. A preparation of a heat-killed *E. coli* strain was radiolabeled with ^{125}I according to the protein labeling method of McConahey and Dixon (18) as previously described (30). The radioactivity was approximately 3.0×10^6 counts per minute (cpm)/ml bacterial suspension.

Measurement of bacterial clearance and organ distribution. After injection of 0.1 ml of ^{125}I -labeled *E. coli* via jugular vein, blood clearance and organ distribution were determined in 4 groups of each 6 animals (immunized and non-immunized sham-operated rats, as well as immunized and non-immunized rats with chronic biliary obstruction). For evaluation of bacterial clearance, 0.5 ml of blood was withdrawn from the femoral vein at 1, 3, 5, 7 and 10 min following challenge. Ten minutes after injection of radiolabeled *E. coli*, the rats were exsanguinated by aortic puncture. After excision of the extrahepatic bile duct, the liver, spleen, lungs and kidneys were removed and weighed. The radioactivity of the samples was determined using a 1260 Multigamma counter (LKB, Stockholm, Sweden), with a background not exceeding 60 cpm. Bacterial clearance rate (K) was determined using the expression of $K = 0.301/T_{1/2}$, where $0.301 = \log_{10}2$ and $T_{1/2}$ is the intravascular half time. The distribution of bacteria in the different organs was expressed in per cent of the injected bacteria, per gram of tissue or in the total organ.

Quantitation of rat IgG antibodies to surface antigens of E. coli. Serum for determination of antibody was taken from 16 rats with biliary obstruction before and 2 weeks after immunization. Antibodies to *E. coli* were determined essentially as described for quantitation of antibodies to group B streptococci (4) using ^{125}I -labeled protein A, which reacts with the Fc-fragments of rat IgG1 and IgG2c (21). In brief, the strain used for immunization was grown overnight in Todd-Hewitt broth at 37 °C, washed three times in PBS (phosphate buffered saline, 0.12 M NaCl, 0.03 M phosphate, pH 7.2) and suspended in 1/10 volume PBS. To 200 μl *E. coli* suspension was added 200 μl PBS containing 0.1 % Tween 20 (Kebo Grave, Stockholm, Sweden) and 100 μl test serum. After incubation at 37 °C for

30 min, 2 ml PBS-Tween was added, the tube was centrifuged at 3000 g for 15 min, and the pellet suspended in 200 μ l PBS-Tween. Radiolabeled protein A, 50 μ l (0.2 μ g) was added, followed by another 2 ml PBS-Tween and centrifugation. The radioactivity in the pellet was then counted and the results expressed in counts per minute (cpm) as the mean of two duplicates. The background of the test, as measured with PBS instead of rat serum was approximately 300 cpm.

Statistical method. Survival rates were evaluated by *Fischer's* exact (probability) test. The results of bacterial clearance and organ distribution were expressed as the mean $\pm$ SEM and the data were evaluated using *Wilcoxon's* test.

RESULTS

Antibody response to vaccination against E. coli in rats with chronic biliary obstruction. Quantitation of IgG antibodies to surface antigens of *E. coli* used for RI challenge demonstrated a more than 20-fold increase in antibodies after vaccination with the homologous strain (Fig. 1).

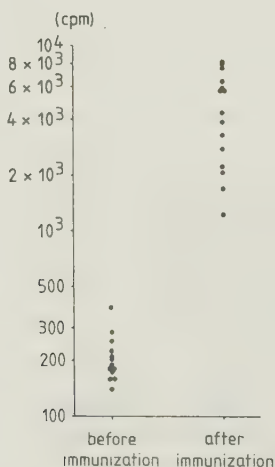


Fig. 1. Quantitation of IgG antibodies to surface antigen of *E. coli* before and after immunization with heat-killed *E. coli* vaccine in rats with chronic biliary obstruction. Quantitation of antibodies was expressed as radioactivity in counts per minute (cpm) (ordinate; log scale).

Lack of protective effect of vaccination against retrograde intrabiliary (RI) challenge with E. coli. RI injection of 10^5 CFU of *E. coli* resulted in a high mortality in the rats with chronic biliary obstruction, irrespective of whether they were vaccinated against the bacteria or not. With the exception of a single rat, all died within 24 h after challenge. Both groups showed significantly higher mortality than the sham-operated rats, of which all survived the RI challenge ($p < 0.0001$; Table I).

Table I. The susceptibility of different groups of rats to retrograde intrabiliary (RI) injection of *E. coli*^a

Groups	Vaccination against <i>E. coli</i>	No. of challenged rats	No. of killed rats
Sham-operation	No	10	0
Obstruction of the common bile duct	No	10	10 ^b
Obstruction of the common bile duct	Yes	10	9 ^b

^a A dose of 10^5 CFU was given.

^b $p < 0.0001$ compared to sham operated rats.

Bile cultures from the rats with chronic biliary obstruction were all negative before bacterial challenge. In contrast, bile as well as blood cultures from all succumbed rats were positive for *E. coli*. Approximately half of the surviving sham-operated rats showed also the presence of *E. coli* in the bile.

Effect of vaccination on blood clearance and organ localization of intravenously injected radiolabeled E. coli. Blood clearance. There were no significant differences in the blood clearance rate for ¹²⁵I-labeled *E. coli* when comparing sham-operated rats and rats with chronic biliary obstruction (K-value in non-immunized and immunized sham operation, and non-immunized and immunized biliary obstruction is, 0.181 ± 0.018 , 0.160 ± 0.005 , 0.167 ± 0.022 , and 0.180 ± 0.008 , respectively).

Organ localization expressed as per cent of injected bacteria per gram tissue. The localization of the bacteria to the liver and spleen was significantly decreased in chronically obstructed rats compared to sham-operated rats ($p < 0.05$; Table II). When vaccinated and non-vaccinated animals with chronic CBD-obstruction were compared, the vaccinated rats demonstrated a significantly higher bacterial trapping in the lungs and kidneys ($p < 0.005$). Among the sham-operated rats, the immunized animals exhibited an increased spleen uptake compared to non-immunized animals.

Organ localization expressed as per cent of injected bacteria in relation to total organ mass. There were no differences between the four groups of animals investigated with respect to liver uptake of bacteria. However, the uptake in the lungs of the animals with chronic biliary obstruction was significantly increased, as compared to sham-operated animals. Furthermore, the immunized rats with chronic biliary obstruction showed significantly higher uptake of the lung than the corresponding non-vaccinated animals (Fig. 2). Finally, the immunized rats with chronic biliary obstruction showed lower uptake of bacteria in the spleen and higher in the kidneys than the sham-operated animals.

Table II. Organ localization per unit weight of intravenously injected ^{125}I -labeled *E. coli* in different groups of rats[§]

Groups	Vaccination against <i>E. coli</i>	Liver	Spleen	Lungs	Kidneys
Sham-operation	No	2.98±0.24	3.51±0.54	0.27±0.02	0.066±0.005
Sham-operation	Yes	3.87±0.08	1.81±0.19 ^a	0.38±0.06	0.073±0.004
3 weeks' obstruction of the common bile duct	No	1.68±0.69 ^b	0.83±0.15 ^b	0.39±0.03 ^a	0.067±0.006
3 weeks' obstruction of the common bile duct	Yes	1.92±0.18 ^b	0.86±0.15 ^b	1.47±0.32 ^c	0.106±0.010 ^c

[§]Values represent mean ± SEM (n = 6 in each group) expressed as per cent of injected bacteria per gm of tissue.

^ap < 0.05 compared to non-immunized sham-operated rats. ^bp < 0.05 compared to both sham operated groups. ^cp < 0.005 compared to all other groups.

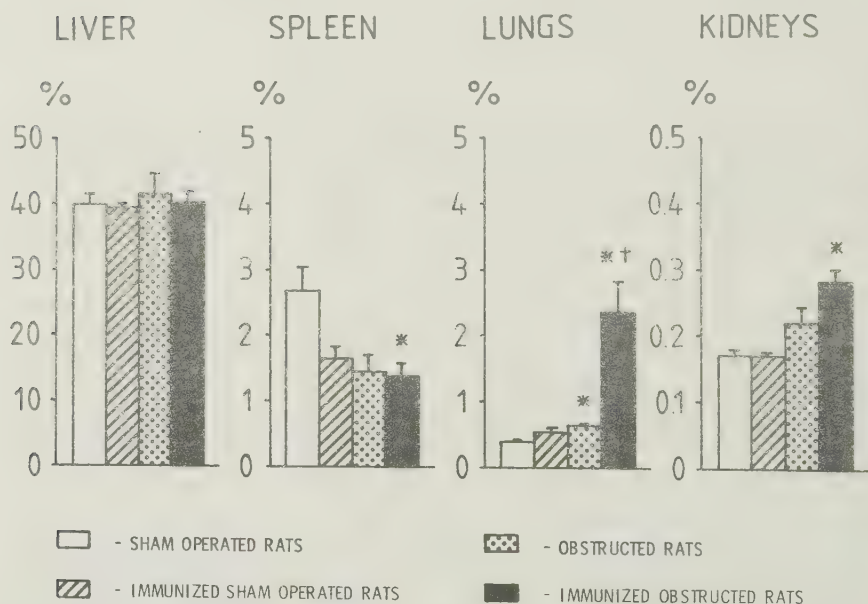


Fig. 2. Organ distribution of intravenously injected ^{125}I -labeled *E. coli* in experimental groups. Values represent mean ± SEM expressed per cent of injected bacteria per total organ. *: p < 0.005 compared to sham-operated, non-immunized rats. †: p < 0.005 compared to non-immunized rats with chronic biliary obstruction.

DISCUSSION

Much attention has recently been focused on immunotherapy for gram-negative bacterial sepsis (2, 6, 31). Since antibiotic treatment is faced with the serious problem of development of bacterial resistance, immunotherapy would seem to be a pertinent adjunct to or replace antibiotics in septic disease. It has been previously shown that immunization of normal animals increases the capacity of the RES to clear bacteria from the blood, and that there is a direct relationship between the amount of specific antibody and the efficacy of phagocytosis of bacteria (1, 26). However, the applicability of these findings in patients with various underlying diseases, including liver diseases as cirrhosis, hepatitis or chronic obstructive jaundice, has not been determined yet.

Obstructive jaundice predisposes to biliary infection. The condition has been suggested to be related to impairment of RES function (8, 10). Since the liver, in the presence of antibodies, is the most efficient organ for trapping of bacteria (9), the main interest in our study was to determine whether active immunization could produce improvement of phagocytosis in chronic biliary obstruction.

The results demonstrated that the vaccination provided a high IgG antibody to surface antigen of *E. coli*. Nevertheless, the enhanced antibody titer did not improve the survival following RI challenge with the same strain. This finding could of course be explained by trivial factors, such as too high doses of *E. coli* for detection of minor differences. However, the results of the clearance studies with radiolabeled *E. coli* might point to other possibilities.

The increased localization of bacteria to the lung in chronic biliary obstruction confirmed a previous report (10). However, the finding that active immunization in rats with chronic biliary obstruction significantly promoted lung uptake of bacteria was unexpected. The mechanism of lung accumulation of bacteria has not been clarified. *Lanser and Saba* (16) reported that the localization of bacteria to the lung following colloid-induced RE blockade was further increased by administration of fibronectin. They suggested that fibronectin increased the number of neutrophils margined in the lung following blockade and thereby provided an increased bacterial uptake in the lung. A similar mechanism of neutrophil-mediated lung localization of bacteria might explain the vaccine-effect. Whatever the mechanism it may be, it seemed plausible that the overloading of the lungs with bacteria following vaccination of rats with chronic biliary obstruction was less beneficial to the animals, thereby neutralizing the advantage of the improved opsonization.

We conclude from the present study that care should be exercised in the use of immunotherapy, such as gammaglobulin, in gram-negative sepsis on patients with chronic liver disease. Further studies are required to determine the possible side effects of such therapy.

REFERENCES

1. Benacerraf, B., Sebestyen, M.M. & Schlossman, S. 1959. A quantitative study of the kinetics of blood clearance of ³²P-labeled *Escherichia coli* and *Staphylococci* by the reticuloendothelial system. *J Exp Med* 110, 27.
2. Braude, A.I., Ziegler, E.J., Douglas, H. & McCutchan, J.A. 1977. Antibody to cell wall cyclo lipid of gram-negative bacteria: Induction of immunity to bacteremia and endotoxemia. *J Infect Dis* 136, 167.
3. Carrasco, C.H., Zornoza, J. & Bechtel, W.J. 1984. Malignant biliary obstruction: Complications of percutaneous biliary drainage. *Radio-logy* 152, 343.
4. Christensen, K.K., Christensen, P., Dahlander, K., Faxelius, G., Jacobson, B. & Svenningsen, N. 1980. Quantitation of serum antibodies to surface antigens of Group B Streptococci type Ia, Ib and III: Low antibody levels in mothers of neonatally infected infants. *Scand J Infect Dis* 12, 105.
5. Drivas, G., James, O. & Wardle, N. 1976. Study of reticuloendothelial phagocytic capacity in patient with cholestasis. *Br Med J* 1, 1568.
6. Dunn, D.L. & Ferguson, R.M. 1982. Immunotherapy of gram-negative bacterial sepsis: Enhanced survival in a guinea pig model by use of rabbit antiserum to *Escherichia coli* J5. *Surgery* 92, 212.
7. Hatfield, A.R.W., Tobias, R., Terblanche, J., Gierwood, A.H., Fa-taar, S., Harries-Jones, R., Kernoff, L. & Marks, I.N. 1982. Pre-operative external biliary drainings in obstructive jaundice. A prospective controlled clinical trial. *Lancet* ii, 896.
8. Holman, J.M. & Rikkers, L.F. 1982. Biliary obstruction and host defense failure. *J Surg Res* 32, 208.
9. Hosea, S.W., Brown, E.J., Hamburger, M.I. & Frank, M.M. 1981. Opsonic requirements for intravascular clearance after splenectomy. *New Eng J Med* 304, 245.
10. Katz, S., Grosfeld, J.L., Gross, K., Plager, D.A., Ross, D., Rosen-thal, R.S., Hull, M. & Weber, T.R. 1984. Impaired bacterial clearance and trapping in obstructive jaundice. *Ann Surg* 199, 14.
11. Keane, R.M., Gadacz, T.R., Munster, A.M., Birmingham, W. & Win-church, R.A. 1984. Impairment of human lymphocyte function by bile salts. *Surgery* 95, 439.
12. Keighley, M.R.B., Lister, D.M., Jacobs, S.I. & Giles, G.R. 1974. *Surgery* 75, 578.
13. Keighley, M.R.B., Drysdale, R.B., Quoraishi, A.H., Burdon, D.W. & Alexander-Williams, J. 1975. Antibiotic treatment of biliary sep-sis. *Surg Clin North Amer* 55, 1379.
14. Keighley, M.R.B., Flinn, R. & Alexander-Williams, J. 1976. Multi-variate analysis of clinical and operative findings associated with biliary sepsis. *Br J Surg* 63, 528.
15. Keighley, M.R.B., Drysdale, R.B., Quoraishi, A.H., Burdon, D.W. & Alexander-Williams, J. 1976. Antibiotics in biliary disease: the relative importance of antibiotic concentrations in the bile and serum. *Gut* 17, 495.
16. Lanser, M.E. & Saba, T.M. 1981. Neutrophil-mediated lung localiza-tion of bacteria: A mechanism for pulmonary injury. *Surgery* 90, 473.

17. Maddocks, A.C., Wilson, G.R.F. & Taylor, R. 1973. The bacteriology of the obstructed biliary tree. *Ann Roy Coll Surg* 52, 316.
18. McConahey, P.J. & Dixon, F.J. 1966. A method of trace iodination of proteins for immunologic studies. *Int Arch Allergy* 29, 185.
19. McPherson, G.A.D., Benjamin, I.S., Habib, N.A., Bowley, N.B. & Blumgart, L.H. 1982. Percutaneous transhepatic drainage in obstructive jaundice: advantages and problems. *Br J Surg* 69, 261.
20. McPherson, G.A.D., Benjamin, I.S., Hodgson, H.J.F., Bowley, N.B., Allison, D.J. & Blumgart, L.H. 1984. Preoperative percutaneous transhepatic biliary drainage: the results of a controlled trial. *Br J Surg* 71, 371.
21. Medgyesi, G.A., Füst, G., Gergely, J. & Bazin, H. 1978. Classes and subclasses of rat immunoglobulins: Interaction with the complement system and with staphylococcal protein. A. *Immunochemistry* 15, 125.
22. Nakayama, T., Ikeda, A. & Okuda, K. 1978. Percutaneous transhepatic drainage of the biliary tract technique and results in 104 cases. *Gastroenterology* 74, 554.
23. Newberry, W.M., Shorey, J.W., Sanford, J.P. & Combes, B. 1973. Depression of lymphocyte reactivity to phytohemagglutinin by serum from patients with liver disease. *Cell Immun* 6, 87.
24. Nilsson, U., Evander, A., Ihse, I., Lunderquist, A. & Mocibob, A. 1983. Percutaneous transhepatic cholangiography and drainage - complications and risks. *Acta Radiologica* 24, 433.
25. Pitt, H.A., Cameron, J.L., Postier, R.G. & Gadacz, T.R. 1981. Factors affecting mortality in biliary tract surgery. *Am J Surg* 141, 66.
26. Schulkind, M.L., Ellis, E.F. & Smith, R.T. 1967. Effect of antibody upon clearance of ^{125}I -labeled pneumococci by the spleen and liver. *Pediat Res* 1, 178.
27. Takada, T., Hanya, F., Kobayashi, S. & Uchida, Y. 1976. Percutaneous transhepatic cholangial drainage: Direct approach under fluoroscopic control. *J Surg Oncol* 8, 83.
28. Tanaka, N., Christensen, P., Rydén, S., Klöfver-Ståhl, B. & Bengmark, S. 1985. A rat model for sepsis in chronic biliary obstructions. *Acta path microbiol scand* in press.
29. Tanaka, N., Christensen, P., Rydén, S., Klöfver-Ståhl, B. & Bengmark, S. 1985. Biliary obstruction and susceptibility to biliary sepsis in rats. *Res Exp Med* in press.
30. Tanaka, N., Christensen, P., Rydén, S., Klöfver-Ståhl, B. & Bengmark, S. 1985. Decreased clearance of *Escherichia coli* from the bile in rats with obstructive jaundice. *Eur Surg Res* in press.
31. Ziegler, E.J., McCutchan, J.A., Fierer, J., Glauser, M.P., Sadoff, J.C., Douglas, H. & Braude, A.I. 1982. Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. *New Eng J Med* 307, 1225.

RETICULOENDOTHELIAL FUNCTION IN RATS WITH OBSTRUCTIVE JAUNDICE

Nobutaka Tanaka, MD, Stefan Rydén, MD, PhD (surg), Lennart Bergqvist, BSci (radiol), Poul Christensen, MD, PhD (microbiol) and Stig Bengmark, MD, PhD (surg)

Departments of Surgery, Microbiology and Radiation Physics, University of Lund, Lund, Sweden

Abstract

Reticuloendothelial (RE) function was evaluated by measuring the bio-kinetics of a standardized $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid in rats with biliary obstruction. When corrected for changes in liver volume, the colloidal uptake rate (cK_1) was significantly decreased in 1 week biliary obstruction ($p < 0.005$ compared to sham operation) and 3 week biliary obstruction ($p < 0.025$ compared to 1 week obstruction). Colloidal uptake rate of the extrahepatic RES was significantly increased ($p < 0.005$) in rats with 3 week biliary obstruction. Activity distribution of $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid in 3 weeks' biliary obstruction was significantly decreased in both total organ basis and per gram basis ($p < 0.005$). The results demonstrated a depression of RE activity of the liver in biliary obstruction.

Infection of the bile and septicemia are well known complications of obstructive jaundice. Earlier findings in our laboratory have shown an increased susceptibility to bacterial infection of the bile duct in experimental animals with bile duct obstruction. Alterations in RE function may be one factor causing the increased mortality in these animals.

The reticuloendothelial system (RES) represents a collection of mesenchymal derived cells which consists of wandering and sessile macrophages. A major portion of the RES is constituted by sessile macrophages located in the liver, spleen, lungs and bone marrow. The macrophages in the liver and spleen manifest approximately 85-95 % of the total intravascular phagocytic capacity (1). Reticuloendothelial (RE) function involves 1) defence against microorganisms, 2) removal of dead or damaged cells, cell debris and inorganic material, 3) regulation of hematopoiesis, 4) cooperative and effector functions in the immune response, and 5) synthesis of biologically active compounds (2).

RE activity has been determined with several types of colloidal preparations (3). Depression of RE activity has been suggested in connection with infection (4-6) and tumor growth (3, 7-9), or after surgery (10), trauma (5, 11, 12) or hemorrhage (13). Using colloidal carbon particles, RE function in obstructive jaundice has been shown to be stimulated (14, 15) or depressed (16). The conflicting results require further studies to evaluate RE function properly in obstructive jaundice.

In previous studies, the efficiency of a scintillation camera technique for measurements of the RE function, using a $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid as the test substance has been investigated (17, 18). The $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid has been well characterized in respect of size, shape, number, and labeling efficiency (19, 21). Dynamic measurements of the biokinetics of a standardized $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid, enable simultaneous studies of the uptake of the colloid by different RE organs.

The aim of the present study was to assess the RE phagocytic ability in rats with biliary obstruction of different duration.

MATERIAL AND METHODS

Animals

Twenty-four male Sprague-Dawley rats weighing 261-370 g were used. The animals were maintained on an artificial day and night cycle and fed laboratory food (pellets) and water ad libitum.

Experimental groups and surgical procedures

All surgical procedures were performed under ether anesthesia using clean but not sterile conditions. Animals were divided into the following three groups. Eight rats (group A) were sham operated (i.e., isolation of the common bile duct without ligation) 1 week before measurement of RE function (one of these rats died just after the measurement of colloidal uptake rate, and was therefore excluded from the further study of organ distribution of colloid, see below). Sixteen rats were

subjected to extrahepatic biliary obstruction by double ligation and excision of the common bile duct between the lowermost tributary of the bile duct and the uppermost tributary of the pancreatic duct 1 week before (group B) and 3 weeks before (group C) measurement of RE function.

Method of determination of RE function

The test substance, $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid ($^{99}\text{Tc}^{\text{m}}_2\text{S}_7$) was prepared according to Persson and Naversten (19). The range of the particle diameter was 200 nm to 500 nm with a mean of 300 nm (21). The number of particles per ml of solution was approximately 10^{10} . The labeling efficiency was tested in each experiment with gel chromatography column scanning technique (20) and was found to be 97-100 %. The technique for dynamic measurements of the colloid distribution has been described earlier (17, 22).

Under ether anesthesia, the jugular vein was cannulated and a catheter was introduced to the superior vena cava. The animals were fixed on a scintillation camera (Maxi Camera, General Electric) equipped with a Leher parallel hole collimator. About 15 MBq in a volume of 0.3 ml test colloid was injected rapidly during 2-4 seconds. Sequential images were taken during 15 min from the start of injection. Three frame groups were stored with 16, 12 and 20 frames at intervals of 7.5, 15 and 30 seconds, respectively. Regions of interest were selected in the image and time activity curves were generated. The curves were stored and transferred to a computer (Digital Equipment PDP 11/45) where the uptake curves were evaluated. To estimate the uptake rates of the radiolabeled colloid to the liver (K_1) and to other RE tissues (K_2), an open two compartment model was used (22). The clearance rate of the colloid from the blood (B) and the rate of uptake in the liver (L) can be written:

$$\frac{dB}{dt} = -B (K_1 + K_2) \quad (1)$$

$$\frac{dL}{dt} = B K_1 \quad (2)$$

The percentage amount of colloid transported to the liver could thus be expressed by the equation:

$$L(t) = 100 \frac{K_1}{K_1 + K_2} \{1 - e^{-(K_1 + K_2)t}\} \quad (3)$$

By fitting this equation to the experimental curves for each animal, the rate of constants K_1 and K_2 were calculated. Due to the pronounced variations of liver weight between the three groups of animals, a correction for these variations was made in analog with a formula originally given by Biozzi for calculation of the corrected phagocytic index (23). The corrected colloidal uptake rate (cK_1) of the liver was thus calculated according to the equation:

$$cK_1 = \sqrt[3]{K_1} \cdot \frac{W}{WL} \quad (4)$$

where W represents the body weight, and WL represents the weight of the liver.

After completion of the registration of the scintillation camera, all animals were exsanguinated by aortic puncture. For measurements of organ activity, the liver, the lungs and the spleen were carefully dissected. Bone marrow aspirates from both femurs were obtained by fine needle aspiration. Each organ or tissue was inserted into a plastic tube, weighed and measured for radioactivity with an automatic sample-changing NaI (Tl) well counter.

Statistical method

The results were expressed as the mean $\pm$ SD and the data were evaluated using Student's t test. A p value of less than 0.05 was considered significant.

RESULTS

Examination of the animals with biliary obstruction revealed in all cases a cystic dilatation of the common bile duct and enlargement of the liver and the spleen. These changes were more pronounced in 3 weeks' biliary obstruction (group C) than in 1 week's obstruction (group B). The relative weight (as a percentage of total body weight) of the liver and spleen was significantly increased ($p < 0.005$) in 1 week's biliary obstruction (group B) compared to sham operation (group A) (Table I). A

Table I. Relative weights (percentage of total body weight) in liver and spleen in rats with biliary obstruction and sham operation^a.

Groups	No. of rats	Liver (%)	Spleen (%)
A. Sham operation	7	3.82 $\pm$ 0.25	0.26 $\pm$ 0.03
B. 1 week obstruction	8	5.34 $\pm$ 0.72 ^b	0.36 $\pm$ 0.06 ^b
C. 3 week obstruction	8	6.71 $\pm$ 0.70 ^c	0.49 $\pm$ 0.09 ^c

^aData are expressed as mean $\pm$ SD

^b $p < 0.005$ compared to sham operation

^c $p < 0.005$ compared to both sham operation and 1 week biliary obstruction

further increase of the relative weight of the liver and spleen was noted in group C compared to group B ($p < 0.005$) (Table I).

The colloid uptake rate in the liver (K_1) was not different between the rats with biliary obstruction and those with sham operation. However, the uptake rate of the extrahepatic RES (K_2) was significantly higher

Table II. Uptake rate of $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid in the liver (K_1) and the extrahepatic RES (K_2) in rats with biliary obstruction and sham operation.

Groups	No. of rats	Colloid uptake rate (s^{-1}) ^a	
		Liver $K_1 \cdot 10^{-1}$	Extrahepatic RES $K_2 \cdot 10^{-2}$
A. Sham operation	8	0.16 ± 0.04	0.15 ± 0.03
B. 1 week obstruction	8	0.15 ± 0.04	0.15 ± 0.09
C. 3 week obstruction	8	0.14 ± 0.03	0.21 ± 0.06^b

^aData are expressed as mean $\pm$ SD

^b $p < 0.005$ compared to both sham operation and 1 week biliary obstruction

($p < 0.005$) in group C than in group A and B (Table II). When the uptake rate was corrected for the increase in liver volume the corrected colloidal uptake rate (cK_1) was significantly decreased in 1 week's biliary obstruction (group B, 4.3 ± 0.8) compared to sham operation (group A, 6.2 ± 0.3) ($p < 0.005$). The decrease of cK_1 was even more prominent in 3 weeks' biliary obstruction (group C, 3.4 ± 0.3) compared to group A ($p < 0.005$) and group B ($p < 0.025$) (Fig. 1).

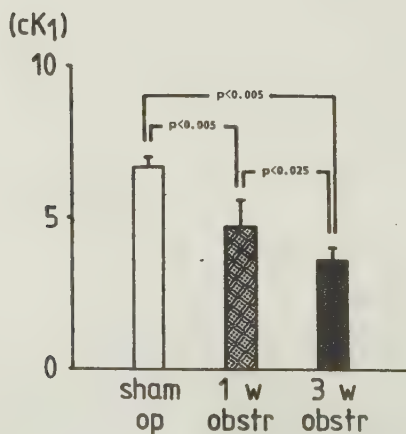


Fig. 1. Corrected colloidal uptake rate (cK_1) in rats with sham operation and biliary obstruction of 1 week and 3 weeks. Bars indicate mean $\pm$ SD.

The total and relative activity distribution of $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid in per cent of injected activity are shown in Table III. The relative

Table III. Activity distribution of $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid in per cent of injected activity in total organ and per gram tissue (mean $\pm$ SD) in rats with biliary obstruction and sham operation.

Groups	Liver		Spleen		Lung		Bone marrow
	%	%/gm	%	%/gm	%	%/gm	%/gm
A. Sham operation	89.5 $\pm$ 4.7	7.4 $\pm$ 0.6	3.7 $\pm$ 2.0	4.4 $\pm$ 2.4	0.39 $\pm$ 0.16	0.29 $\pm$ 0.12	0.17 $\pm$ 0.05
B. 1 week obstruction	89.4 $\pm$ 3.7	6.0 $\pm$ 1.0 ^a	3.4 $\pm$ 0.9	3.4 $\pm$ 0.8	0.54 $\pm$ 0.22	0.41 $\pm$ 0.16	0.18 $\pm$ 0.05
C. 3 week obstruction	83.3 $\pm$ 4 ^b	3.7 $\pm$ 0.7 ^b	4.7 $\pm$ 1.2 ^c	2.8 $\pm$ 0.7	0.98 $\pm$ 0.86	0.63 $\pm$ 0.52	0.30 $\pm$ 0.07 ^b

^a $p < 0.005$ compared to sham operation (group A)

^b $p < 0.005$ compared to both sham operation (group A) and 1 week biliary obstruction (group B)

^c $p < 0.025$ compared to 1 week biliary obstruction (group B)

activity in the liver was decreased in group B compared to group A ($p < 0.005$). The decrease of liver activity per gram tissue was even more pronounced in group C ($p < 0.005$). Total organ uptake rate of the liver in this group was also significantly decreased compared to groups A and B ($p < 0.005$). There were no significant differences in the activities of the spleen and lungs per gram tissue between the three groups, although total organ uptake in the spleen was significantly increased in group C compared to group B ($p < 0.025$). A significant increase of bone marrow activity per gram tissue was noted in group C compared to group A and B ($p < 0.005$).

DISCUSSION

In the present study, colloidal uptake rate of the liver (K_1) in the rats with biliary obstruction was not different from that of sham operated rats. Particle ingestion by RE cells has been suggested to be influenced by blood flow (24) and opsonins (1). Experimental studies of hemodynamics in biliary obstruction have shown an increase of liver blood flow 1 week after biliary obstruction but a decrease of blood flow 3 weeks after obstruction (25). The role of opsonins in biliary obstruction has not yet been clarified. Thus, the contribution of these two factors in the present data of colloidal uptake rate is hard to assess.

On the other hand, the corrected colloidal uptake rate (cK_1) was found to be significantly decreased already one week after biliary obstruction. The longer the period of biliary obstruction, the more depressed the RE function as estimated by cK_1 . Corrected colloidal uptake rate (cK_1) in the present study corresponds to the corrected phagocytic index α first described by Biozzi to enable comparison between animals with different liver and spleen volumes (23). Prolonged biliary obstruction has been known to induce liver enlargement and hypersplenism (16). This splenic growth might be due to Kupffer cell dysfunction, causing antigens to gain access to the spleen, or due to a secondary portal hypertension (26, 27), which diverts portal antigens to systemic circulation.

The results of the corrected colloidal uptake rate in the present study were in contrast to previous studies of Halpern et al (14), who showed an increased activity of RES until 5 weeks of biliary obstruction. However, Holman and Rikkers (16) detected RE depression 3 weeks after biliary obstruction. In the present study a depression of RE function was suggested as early as 1 week after biliary obstruction. The diverse results might be ascribed to the differences in physico-chemical properties of the test substance used, or to the experimental procedures for producing biliary obstruction. In the present study, biliary obstruction was created by excision of the segment of the common bile duct in order not to promote recanalization of the bile duct (28). Thus, rats with 1 week's biliary obstruction already showed enlargement of the liver with cystic dilatation of the common bile duct. Another explanation for the diverse results can be the differences in the technique for a measurement of colloidal uptake rate. Suppression of colloid uptake in the liver is often accompanied by an increased colloid uptake in the extrahepatic RES. However, a classical technique of plasma colloid clearance does not permit separate studies of the uptake by different RE organs. The corrected phagocytic index using plasma clearance rate of colloid in the earlier studies (14, 16) therefore might have masked a depression of RE function in the liver.

The technique applied in the present study permits separate studies of hepatic and extrahepatic (mainly spleen) RE function. Thus, using an open two-compartment model, we could subtly detect a change in RE function of the liver as early as 1 week after biliary obstruction.

The colloidal uptake rate of the extrahepatic RES (K_2) was shown to be increased in 3 weeks' biliary obstruction. This is in accordance with the results of activity distribution of $^{99}\text{Tc}^{\text{m}}$ -sulphur colloid per gram tissue, which showed a decreased activity in the liver and an increased activity in the bone marrow 3 weeks after biliary obstruction. A compensation for hepatic RE depression has been shown to be related to an increase of particle accumulation in the spleen (7, 18), lungs (12) and bone marrow (29). In the present study a tendency of increased accumulation of particles in the lung was noted; however, there were no statistically significant differences between the three groups. This may be due to the great variance of data. Since smaller particles are known to accumulate in the bone marrow and larger particles are readily trapped in the lung (29), the activity distribution in the organism is influenced by the size of particles. The present results were obtained with the use of a highly standardized colloidal preparation. Thus, variation in colloidal particle size should not be an explanation for the differences in colloidal distribution.

The total organ colloid uptake reflects the functioning mass of macrophages in the corresponding organ. Thus, the decreased colloidal uptake in the liver as a total organ strongly suggests a depression of the hepatic macrophage function in 3 weeks' biliary obstruction. Earlier studies of biliary sepsis in connection with biliary obstruction in rats have shown an increased mortality and a decreased hepatic clearance of bacteria after introduction of bacteria in the bile duct (30, 31). According to the present results, it is quite likely that the increased

susceptibility to infection in obstructive jaundice is related to hepatic RE depression as a result of longstanding biliary obstruction. On the other hand, signs of depressed liver macrophage function were not evident in 1 week's biliary obstruction, as judged by extrahepatic colloidal uptake or total organ uptake. Although earlier studies in our laboratory have also shown an increased susceptibility to infection of the bile one week after biliary obstruction (32), it is thus still uncertain whether a depressed RE function of the liver exists already one week after biliary obstruction.

In conclusion, a decreased corrected liver colloid uptake rate as well as a decreased total liver uptake and an increased uptake in the extrahepatic RES strongly suggest a depression of liver RE function. The results may warrant early decompression of the bile ducts in obstructive jaundice. The effect of such decompression on RE function will be elucidated in further studies.

REFERENCES

1. Saba TM: Physiology and physiopathology of the reticuloendothelial system. *Arch Intern Med* 1970; 126:1031-1052
2. Tanner AR, Arthur MOP, Wright R: Macrophage activation, chronic inflammation and gastrointestinal disease. *Gut* 1984; 25:760-783
3. Rydén S: Reticuloendothelial function. Methods to evaluate function and correlation between function and tumor growth - experimental studies in the rat. Thesis, Lund University 1983
4. Biozzi G, Halpern BN, Benacerraf B, Mouton D: Phagocytic activity of the reticuloendothelial system in experimental infections. In: Halpern BN (ed): *Physiology of the reticuloendothelial system*. CC Thomas, Springfield, Ill, 1957; 204-225
5. Kaplan JE, Scovill WA, Bernard H: Reticuloendothelial phagocytic response to bacterial challenge after traumatic shock. *Circ Shock* 1977; 4:1-
6. Lanser ME, Saba TM: Opsonic fibronectin deficiency and sepsis. Cause or effect? *Ann Surg* 1982; 195:340-345
7. Old LJ, Clarke DA, Benacerraf B, Goldsmith M: The reticuloendothelial system and the neoplastic process. *Ann NY Acad Sci* 1960; 88: 264-280
8. Levy MH, Wheelock EF: The role of macrophage in defense against neoplastic disease. *Adv Cancer Res* 1974; 20:131-163
9. DiLuzio NR: Macrophage functional status and neoplasia. In: Altura BM, Saba TM (eds): *Pathophysiology of the reticuloendothelial system*. Raven Press, New York, 1981; 209-224
10. Saba TM: Mechanism mediating reticuloendothelial system depression after surgery. *Proc Soc Exp Biol Med* 1970; 133:1132-1136
11. Saba TM: Effect of surgical trauma on the clearance and localization of blood-borne particulate matter. *Surgery* 1972; 71:675-685
12. Saba TM: Reticuloendothelial systemic host defense after surgery and traumatic shock. *Circ Shock* 1975; 2:91-108
13. Zweifach BW, Benacerraf B: Effect of hemorrhagic shock on the phagocytic function of Kupffer cells. *Circ Res* 1988; 6:83-87
14. Halpern BN, Biozzi G, Nicol T, Bilbey DLJ: Effect of experimental biliary obstruction on the phagocytic activity of the reticuloendothelial system. *Nature* 1957; 180:503-504
15. Lasar G: Influence of splenectomy, partial hepatectomy and bile duct ligation on the granulopoietic activity of the reticuloendothelial system. *Acta Physiol Acad Sci Hung* 1972; 42:287-291
16. Holman JM, Rikkers LF: Biliary obstruction and host defense failure. *J Surg Res* 1982; 32:208-213
17. Rydén S, Strand SE, Palmer J, Stenram U, Hafström L, Persson B: A scintillation camera technique for measurements of the reticuloendothelial function - comparison of different methods for measuring RES function. *Eur J Nucl Med* 1982; 7:16-21
18. Rydén S, Bergqvist L, Hafström L, Strand SE: Reticuloendothelial function in normal and tumor-bearing rats. Measurements with a scintillation camera technique. *Eur J Clin Oncol* 1983; 19:965-970
19. Persson RBR, Naversten Y: Technetium-99m sulfide colloid preparation for scintigraphy of the reticuloendothelial system. *Acta Radiol Ther Phys Biol* 1970; 9:567-576

20. Persson RBR, Strand SE, Knöös T: Radioanalytical studies of ^{99m}Tc -labelled colloids and macromolecules with gel chromatography column scanning technique. *J Radioanal Chem* 1978; 43:275-286
21. Bergqvist L, Strand SE, Persson B: Particle sizing and biokinetics of interstitial lymphoscintigraphic agents. *Semin Nucl Med* 1983; 13:9-19
22. Rydén S, Bergqvist L, Hafström L, Hultberg B, Stenram U, Strand SE: Influence of a reticuloendothelial suppressing agent on liver tumor growth in the rat. *J Surg Oncol* 1984; 26:245-251
23. Biozzi G, Benacerraf B, Halpern BN: Quantitative study of the granulopoietic activity of the reticuloendothelial system. II; A study of the kinetics of the granulopoietic activity of the RES in relation to the dose of carbon injected. Relationship between the weight of the organ and their activity. *Br J Exp Path* 1953; 34:441-457
24. Grün M, Broelsch ChE, Wolter J: Influence of portal hepatic blood flow on RES function. In: *The reticuloendothelial system and the pathogenesis of liver disease*. Elsevier, Amsterdam, 1980; 149-158.
25. Aronsen KF, Nylander G, Ohlsson EG: Liver blood flow studies during and after various periods of total biliary obstruction in the dog. *Acta Chir Scand* 1969; 135:55-59
26. Ohlsson EG, Rutherford RB, Boitnoit JK, Haalebos MMP, Zuidema GD: Changes in portal circulation after biliary obstruction in dogs. *Am J Surg* 1970; 120:16-22
27. Franco D, Gigou M, Szekely, Bismuth H: Portal hypertension after bile duct obstruction. Effect of bile diversion on portal pressure in the rat. *Arch Surg* 1979; 114:1064-1067
28. Trams EG, Symeonidis A: Morphologic and functional changes in the livers of rats after ligation or excision of the common bile duct. *Am J Path* 1957; 33:13-27
29. Atkins HL, Hauser W, Richards P: Factors affecting distribution of technetium-sulphur colloid. *J Reticuloendo Soc* 1970; 8:176-184
30. Tanaka N, Christensen P, Rydén S, Klöfver-Ståhl B, Bengmark S: A rat model for sepsis in chronic biliary obstruction. *Acta Path microbiol scand* 1985; in press
31. Tanaka N, Christensen P, Rydén S, Klöfver-Ståhl B, Bengmark S: Decreased clearance of *Escherichia coli* from the bile in rats with obstructive jaundice. *Eur Surg Res* 1985; in press
32. Tanaka N, Christensen P, Rydén S, Klöfver-Ståhl B, Bengmark S: Biliary obstruction and susceptibility to biliary sepsis in rats. *Res Exp Med* 1985; 185:115-119

PRINTING
ILLUSTRATION DEPARTMENT
DEPARTMENT OF SURGERY
UNIVERSITY OF LUND
1985.